Vaccines for Seasonal and Pandemic Influenza

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Development of licensed influenza vaccines in the United States

1933 – Influenza transmitted to humans

1936 – Demonstration that SC injection of inactivated virus protects animals

1937 – Use of live vaccine suggested by Smoridintsef

1943 – 70% efficacy of inactivated vaccine demonstrated in humans

1945 – Inactivated vaccine licensed in US

1966 – Introduction of the zonal centrifuge

1966 – Cold-adapted influenza virus developed by Massaab

1975 – Content of inactivated vaccine standardized by SRID test

1995 – Cold-adapted vaccine shown to have >95% efficacy in children

2003 – Cold-adapted vaccine licensed for 5 – 49 yo in US

Major issues in influenza vaccination

- Vaccine supply
  - Cell culture vaccines
  - Recombinant vaccines
- Improved efficacy
  - Higher doses
  - Addition of adjuvants
  - Live vaccines
  - DNA vaccines
- Effective pandemic vaccination

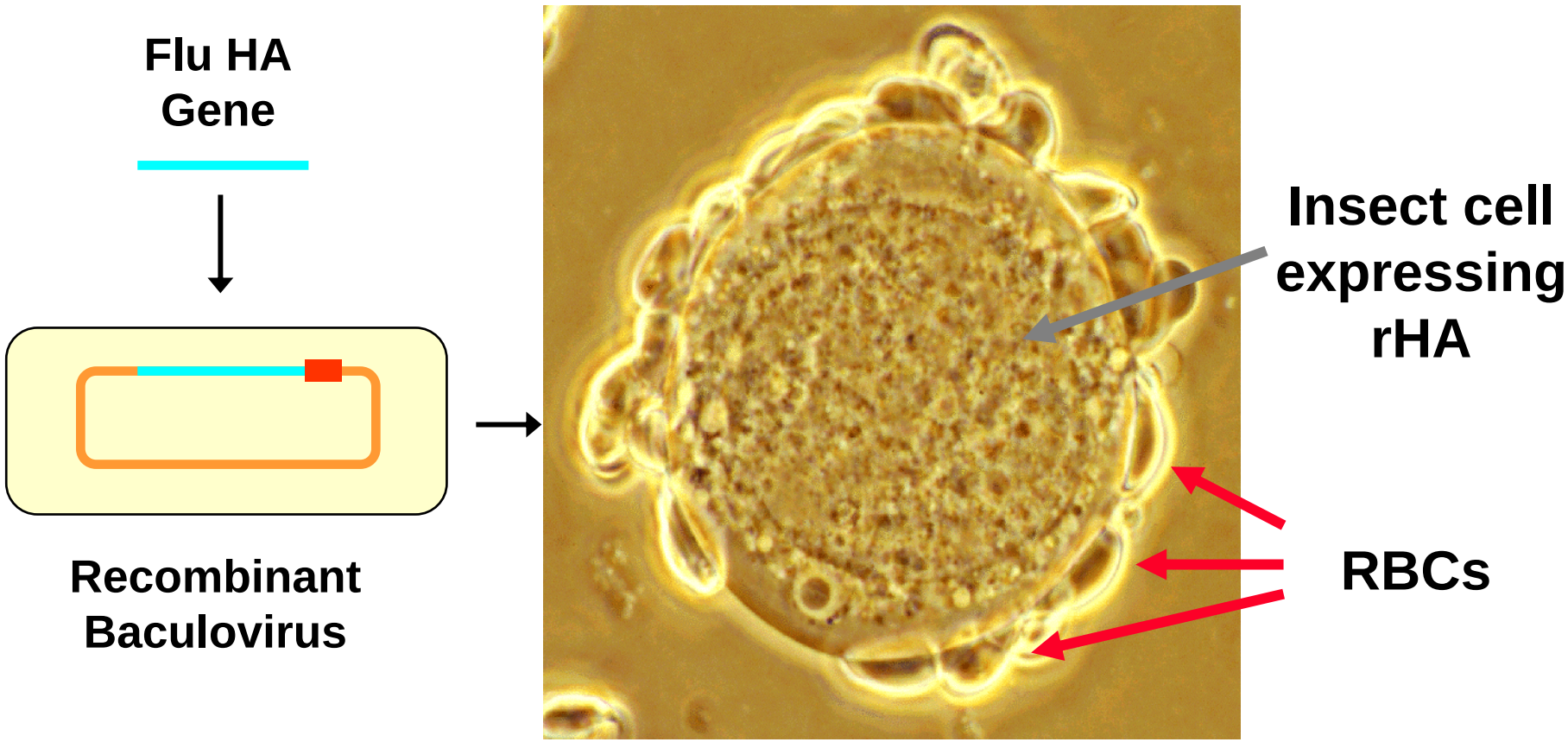
Problems with egg based production

- Specialized and unique production
- Could be difficult to increase supply if needed
- Vulnerable population in event of outbreaks of avian disease
- Easy to contaminate
- Selection of receptor variants
- Egg allergy

Alternatives to egg based production of inactivated influenza vaccines

- Production in cell culture
  - **MDCK**: Canine epithelial cells
  - **Per.C.6**: Adenovirus transformed human conjunctival cells
  - **Vero**: Monkey kidney epithelial cells
  - Generally, trade off between high levels of production but more difficulty certifying lines (MDCK) and lower levels of production in certified lines (Vero)
- Expression of recombinant antigens
  - Insect cell/baculovirus hemagglutinin (rHA)
  - Insect cell/baculovirus VLP (HA, NA, M)

Recombinant baculovirus influenza vaccine (rHA_0, FluBlØk)



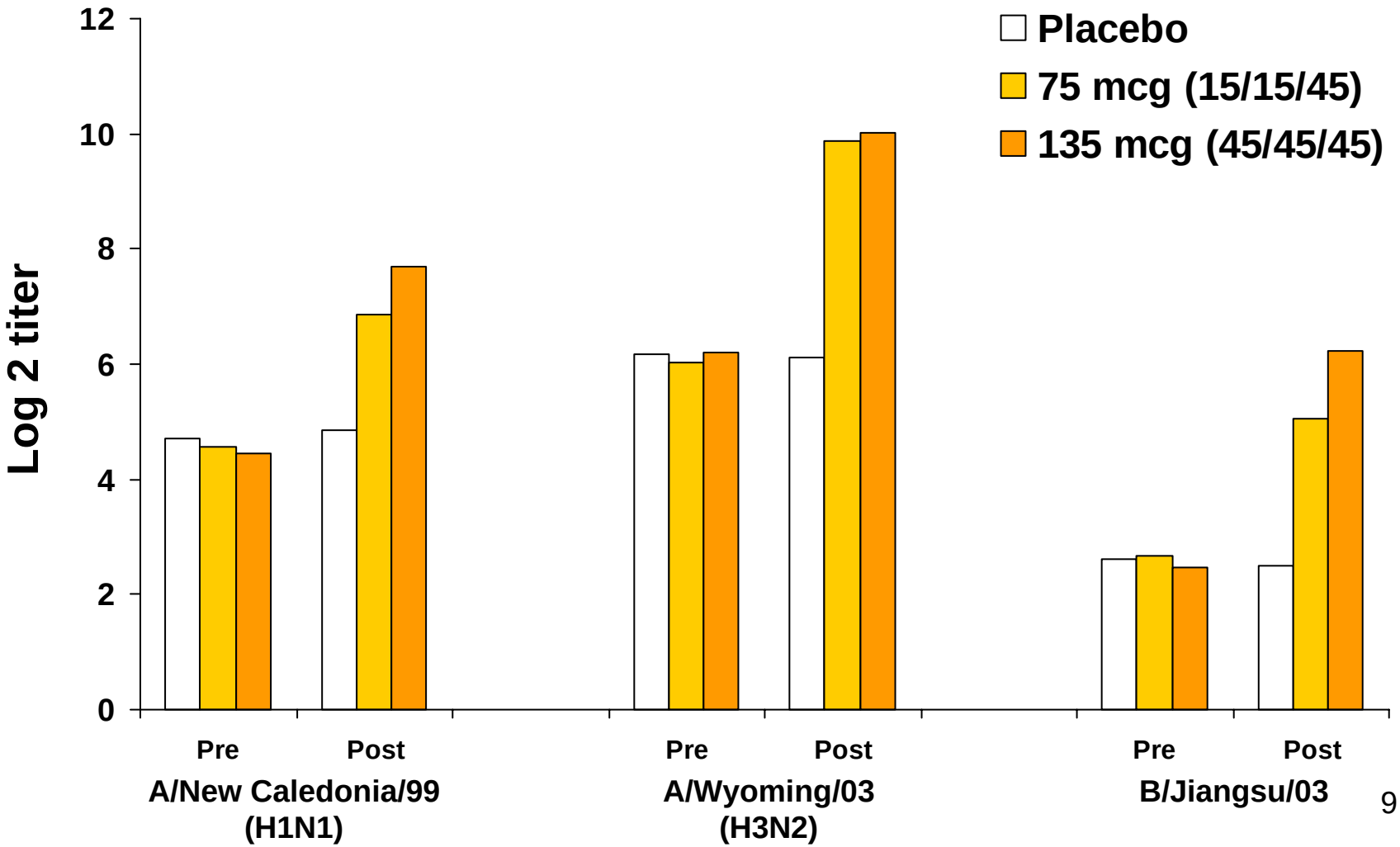
Clinical development of rHA_0 vaccines

- Induces HAI and neutralizing antibody in healthy adults (H3)
- Monovalent preparation showed possible protective efficacy (H3)
- No interference between components of bivalent (H1 + H3 vaccine)
- Well tolerated at doses up to 135 mcg in elderly, immunogenic (H3)
- Improved antibody responses in elderly subjects when administered at high dose (Trivalent)

PSC01 Study design

- Objectives
  - Establish final dose using SRID as potency assay
  - Preliminary demonstration of protective efficacy
- Study design
  - Healthy adults ages 18-49
  - Randomized to trivalent rHA0 vaccine 1:1:1
    - 75 mcg (15 mcg B, 15 mcg H1, 45 mcg H3) *n=150*
    - 135 mcg (45 mcg B, 45 mcg H1, 45 mcg H3) *n=150*
    - Placebo *n=151*
  - Safety: memory aids, solicited and unsolicted AEs
  - Immunogenicity: Day 0 and 28 serum HAI
  - Efficacy: Lab confirmed CDC-ILI

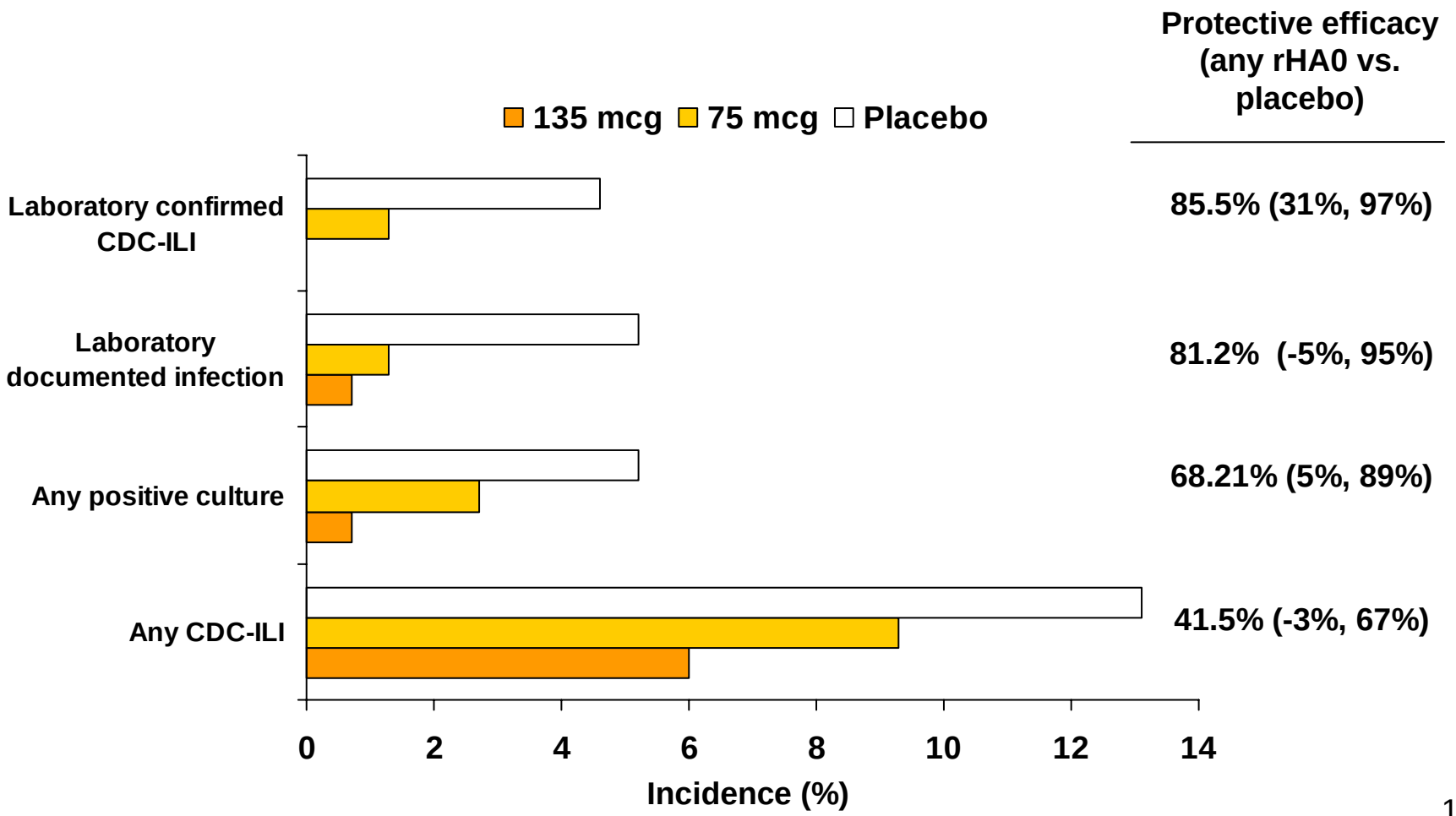
Serum antibody response to trivalent rHA0 vaccine in healthy adults



Influenza activity 2004-2005

- 13 positive cultures
  - 3 influenza B
  - 10 influenza A (all H3N2 viruses)
- All H3N2 study isolates genetically resemble antigenically drifted A/Cal/7/2004 (75% of all US H3N2 isolates)
- 9/13 (69%) of culture positive cases met ILI definition

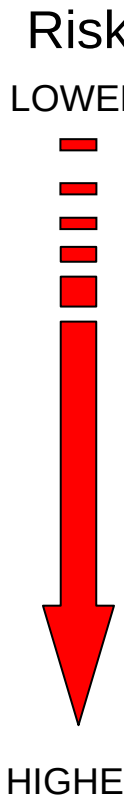
Protective efficacy of rHA_0 in adults



rHA0 conclusions/status

- Dose response relationship demonstrated for all three components
- 45 mcg per component, as determined by SRID, chosen for further evaluation
- 45 mcg dose has protective efficacy against H3N2 influenza
- Protection against an antigenically drifted strain demonstrated in absence of NA component of vaccine

Options for Pandemic Vaccines



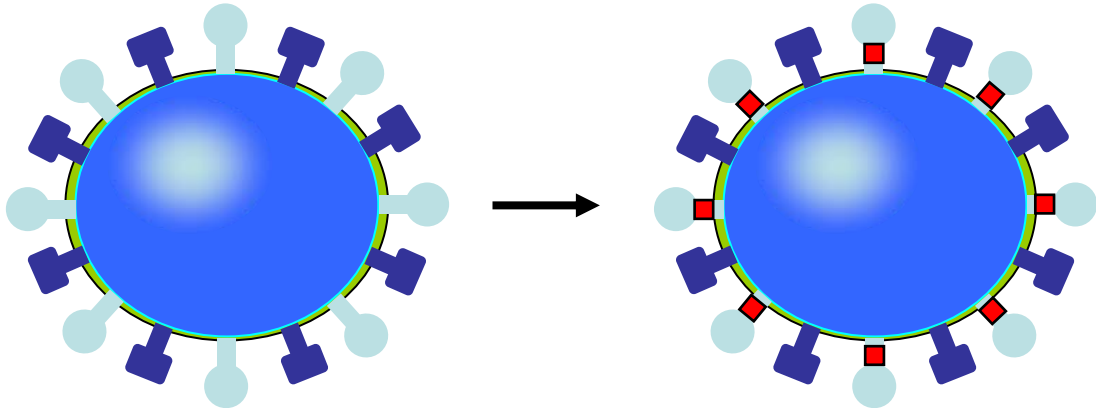
- Inactivated vaccine resembling currently licensed inactivated vaccine (+/- licensed adjuvant)
- Live vaccine resembling currently licensed live vaccine
- Inactivated vaccines with experimental adjuvants/route of administration
- Experimental approaches (DNA vaccines, peptides, vectors)

Strategies for production of vaccine seed viruses

- Problem: HPAIV are lethal for eggs, must be used under high levels of containment
  - Use antigenically related LPAIV (e.g., Duck/Singapore/97, H5N3)
  - Use expressed recombinant protein (e.g., rHA A/HK/156/97)
  - Use reverse genetics techniques to alter HA cleavage site (e.g., rg A/VN/1203/04 x PR8)

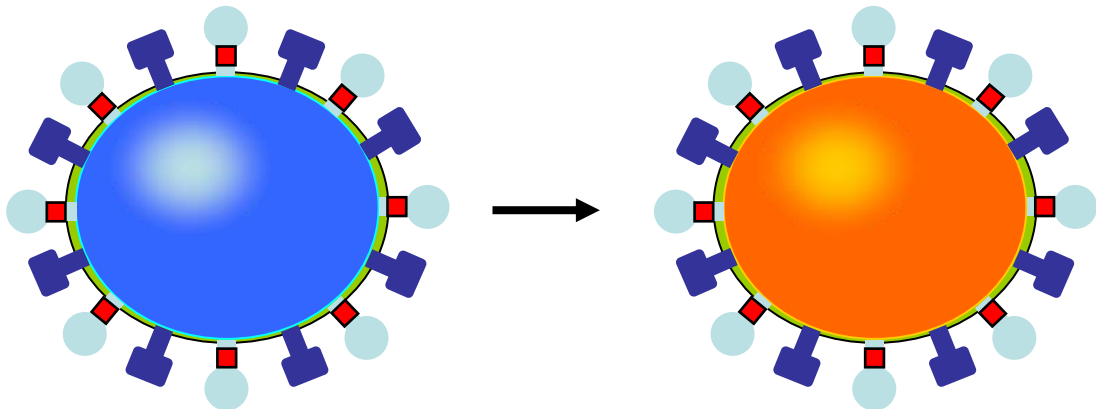
sanofi pasteur H5N1 vaccine virus

1. Engineer the cleavage site



A/Vietnam/1203/04

2. Change internal genes to PR8

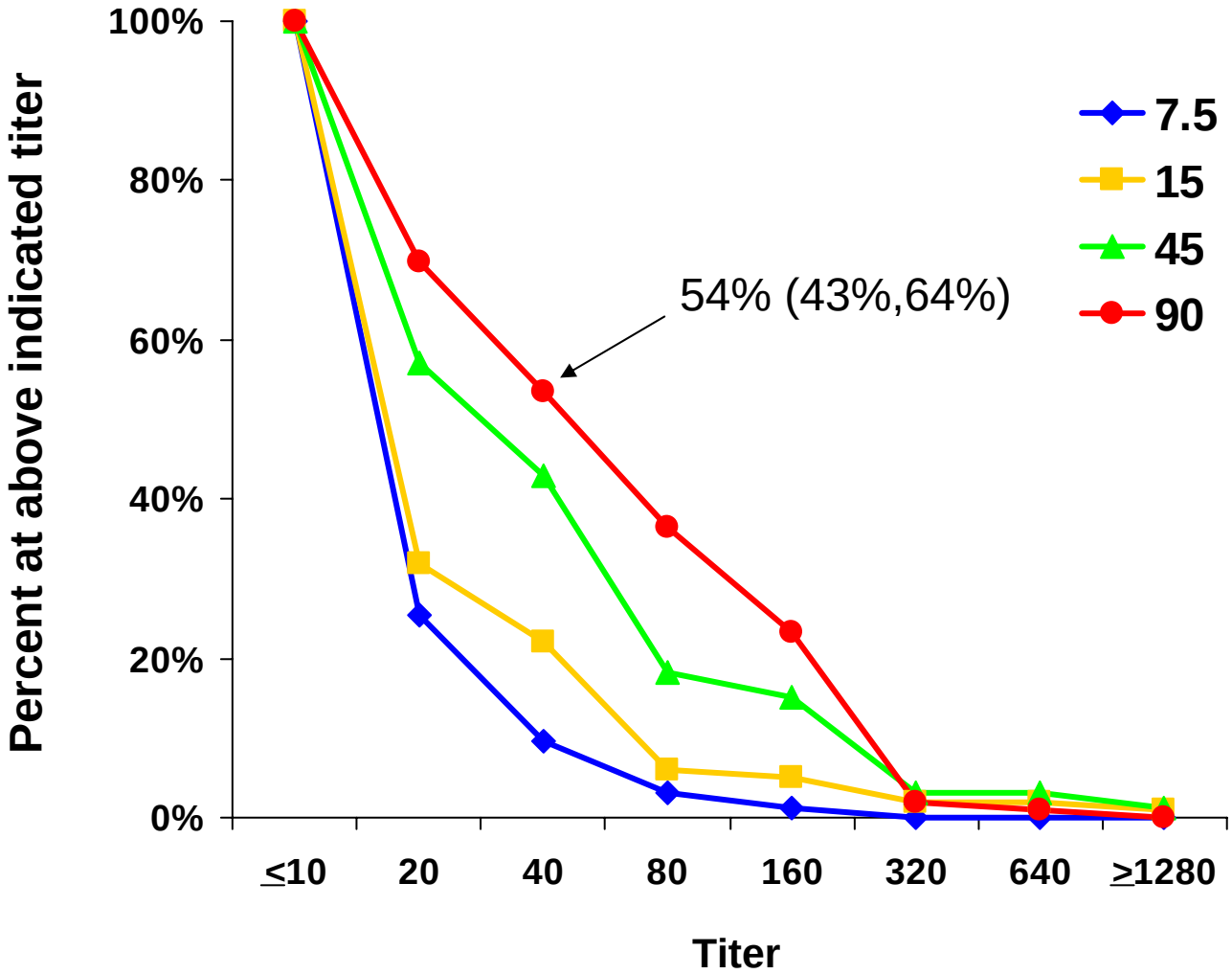


rgA/Vietnam/1203/04xPR8

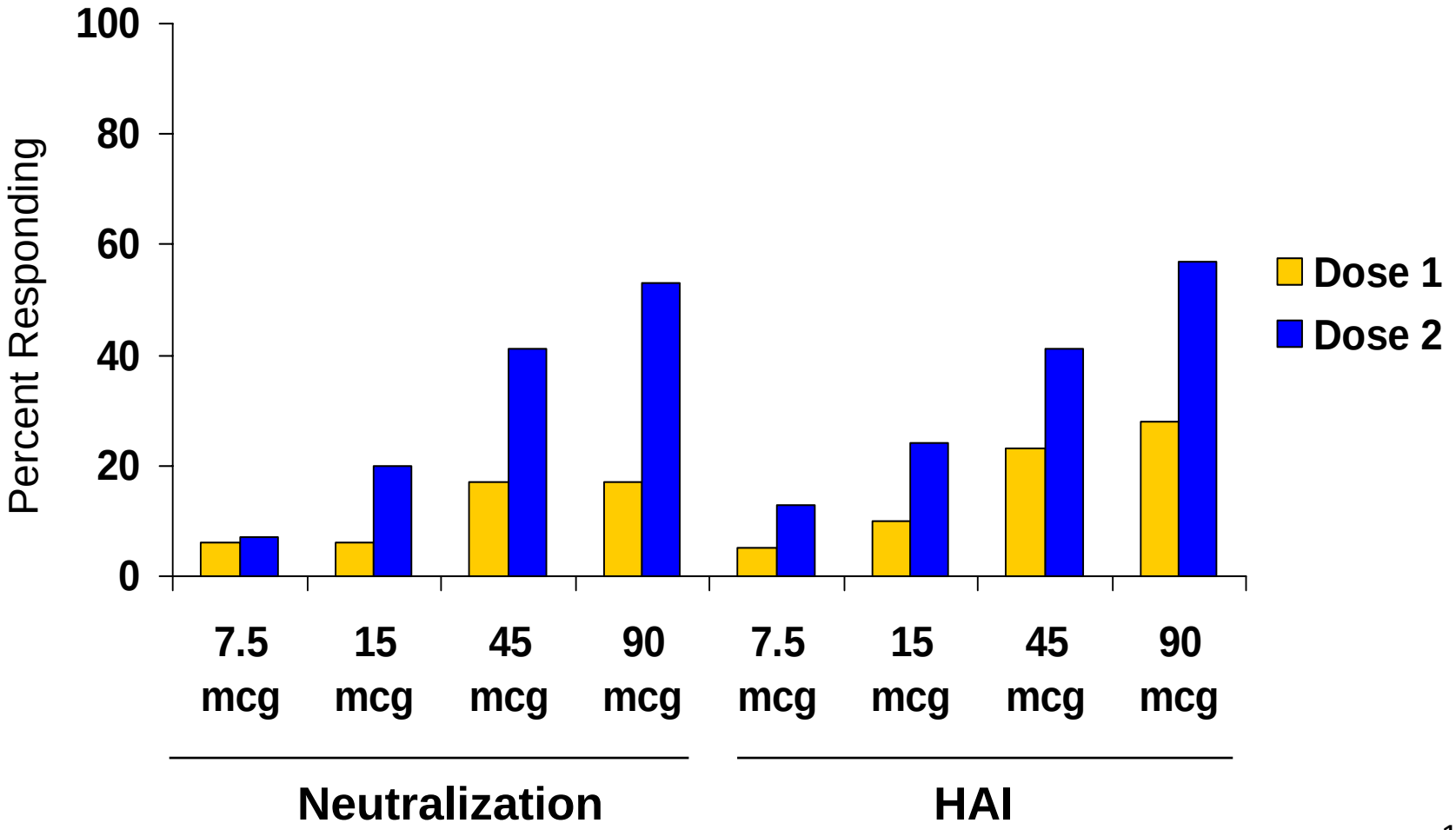
Initial evaluation of H5: DMID 04-063

- Subjects: Healthy adults ages 18 to 64
- Design: Prospective, multicenter, randomized, double blind clinical trial
- Interventions: Two IM doses H5 vaccine separated by 28 days
  - Placebo, 7.5 mcg, 15 mcg, 45 mcg, 90 mcg
  - 1:2:2:2:2 randomization
- Endpoints
  - Safety: solicited and unsolicited AEs
  - Immunogenicity: neutralizing (MN) and HAI antibody
  - Primary endpoint was proportion achieving MN titer of $\geq$ 1:40, HAI was also analyzed.

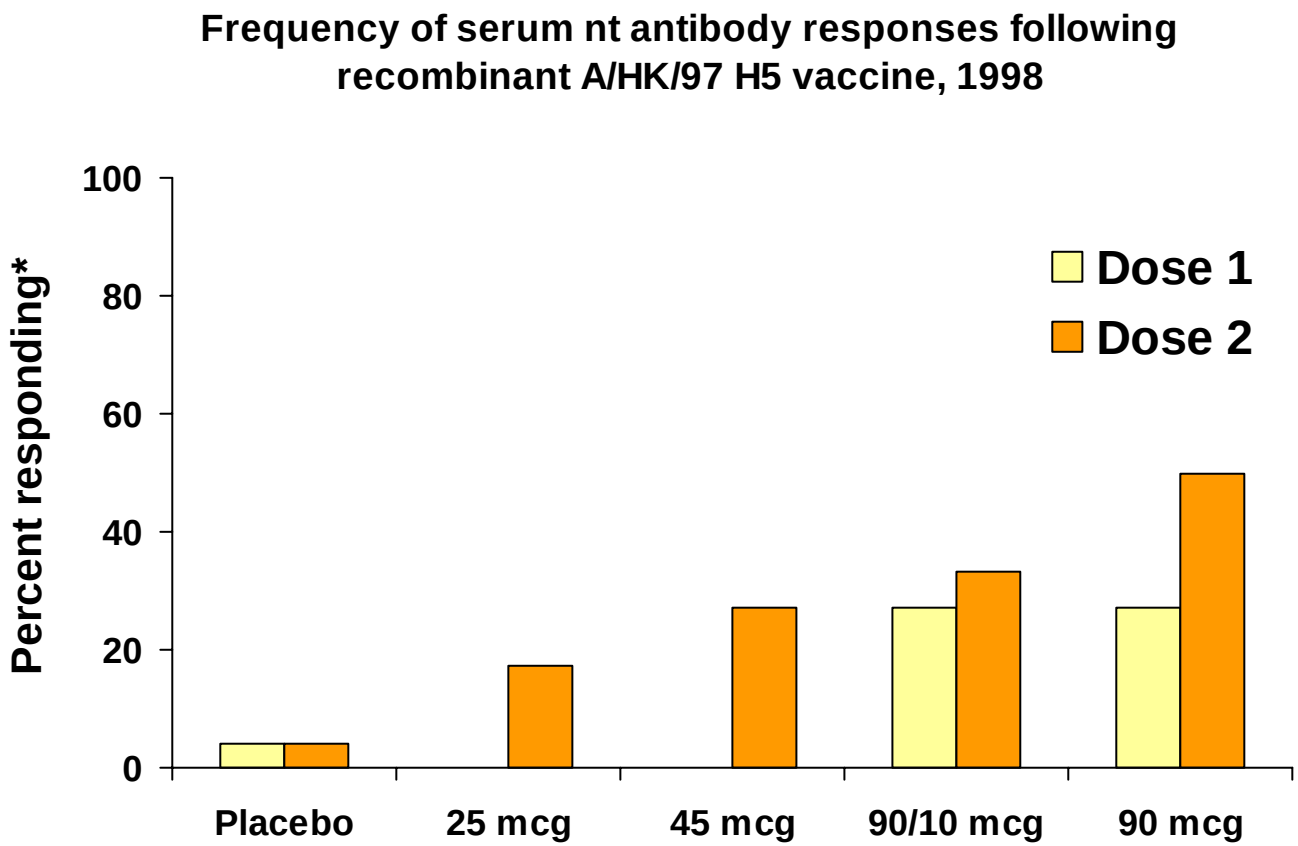
Reverse cumulative distribution of serum MN titers after two doses



Antibody response (4-fold or greater titer increase)



Responses to A/VN/1203/04 subvirion vaccine were similar to A/HK/156/97 rHA vaccine



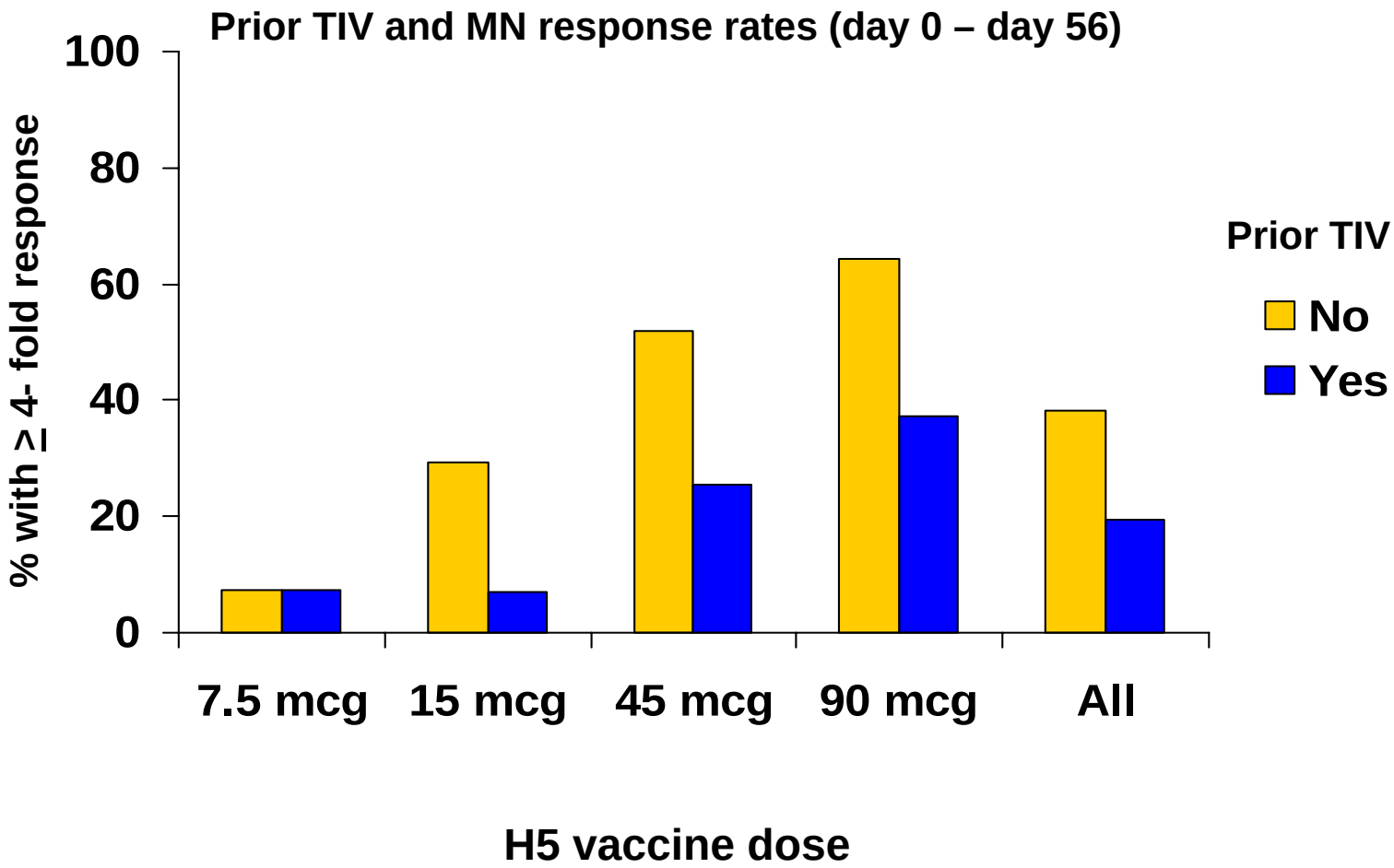
* 4-fold or greater increase to a titter of 1:80 with positive WB

Vaccine 19:1732, 2001

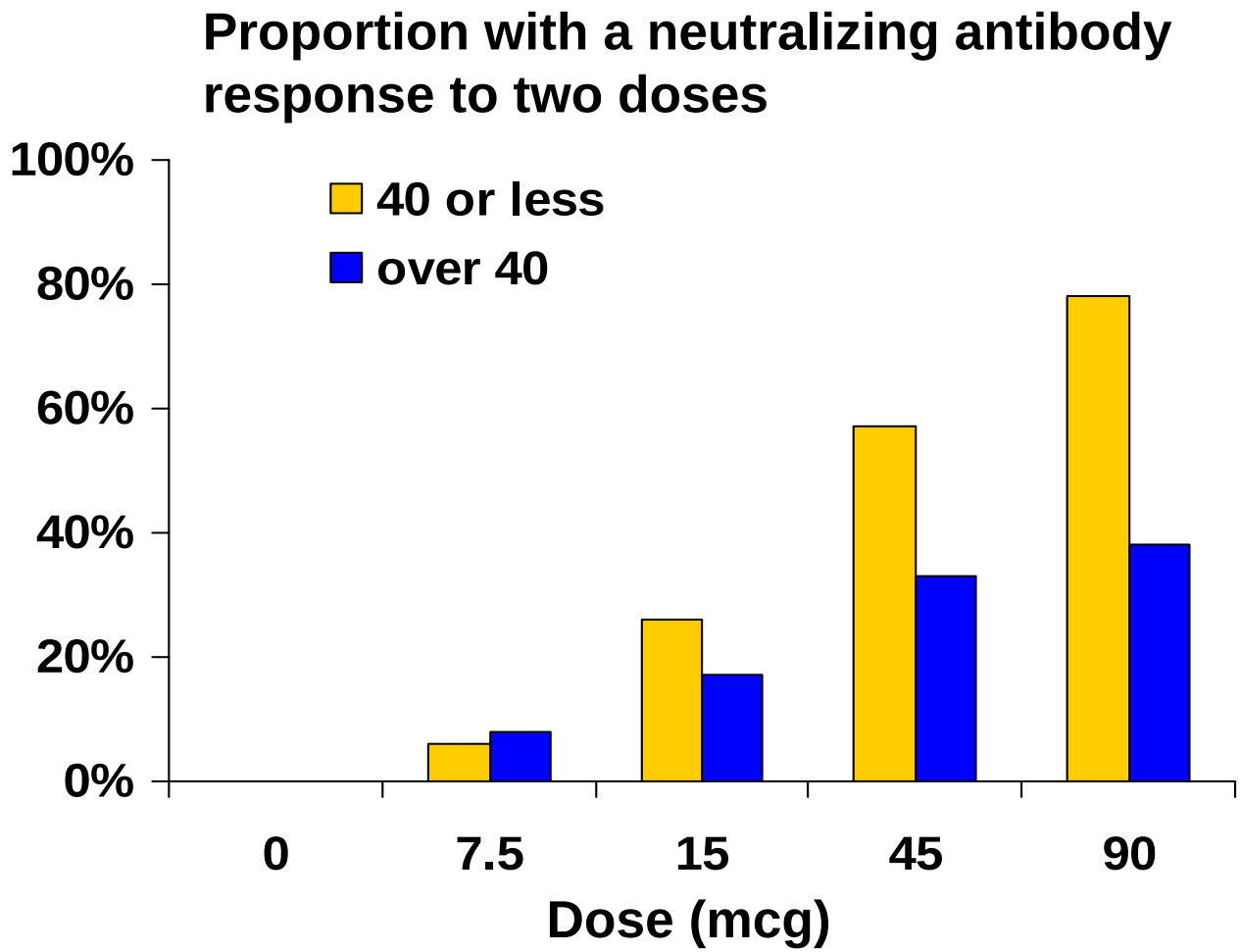
Factors affecting response rates

- Receipt of TIV in the previous fall (significantly lower responses)
- Age > 40 years (significantly lower responses)
- Male (significantly lower rates)
- Multivariate analysis pending

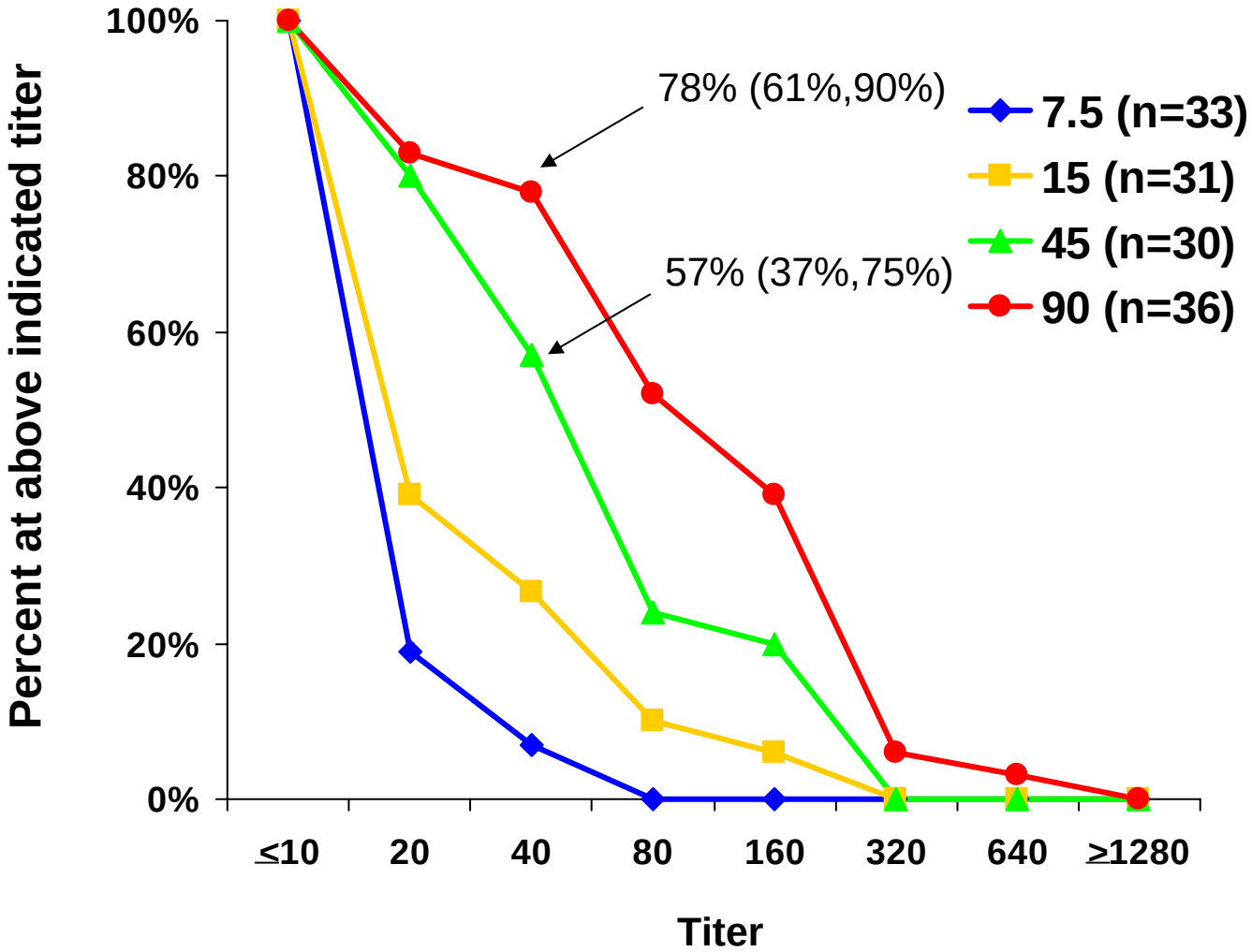
Subjects who received TIV in the fall of 2004 had lower response rates to H5 vaccine in spring 2005



Subjects over 40 had lower response rates than subjects 40 or less



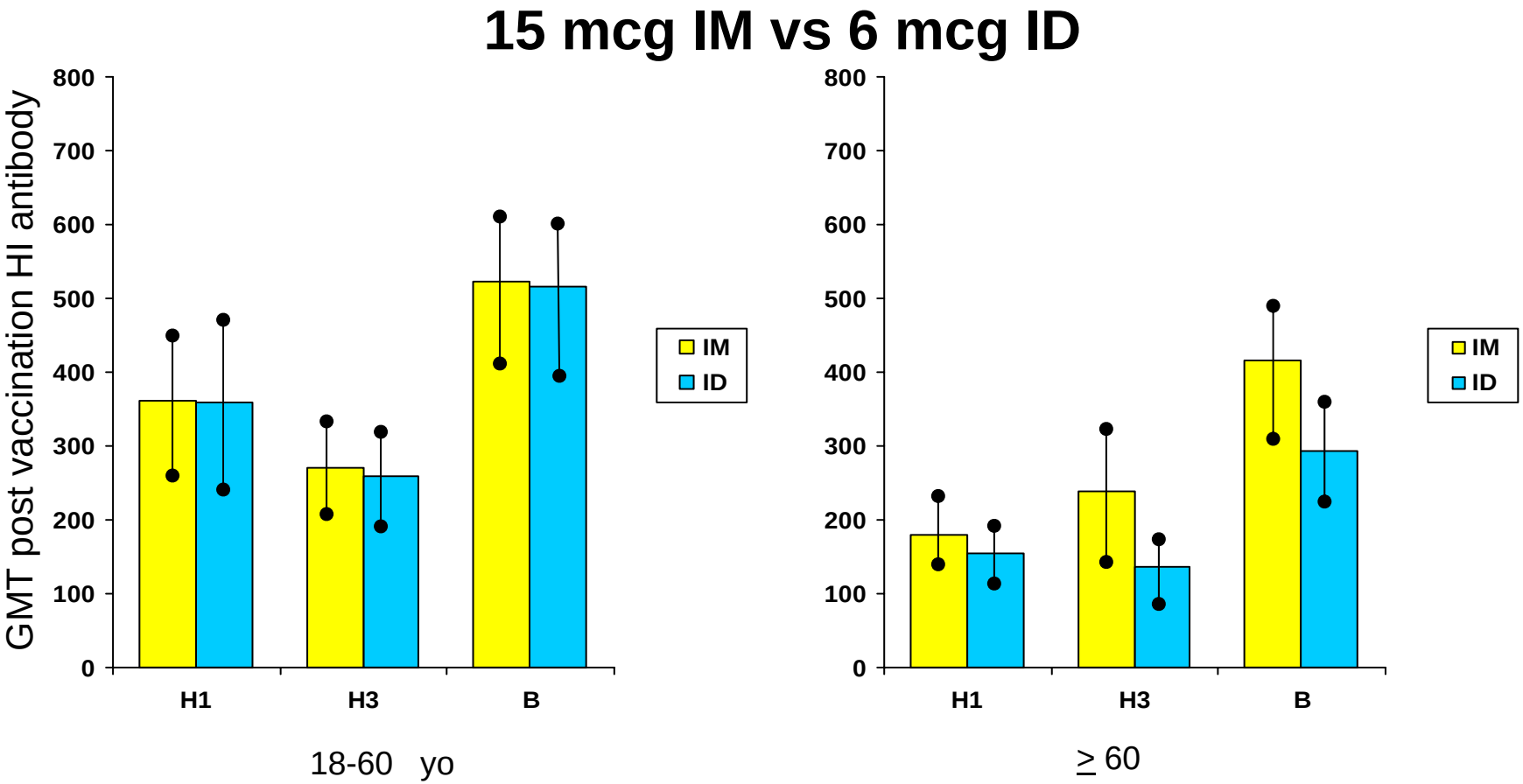
Subgroup analysis: Subjects 18-40 with no hx of TIV, MN RCD



Strategies towards improved vaccination against pandemic infuenza

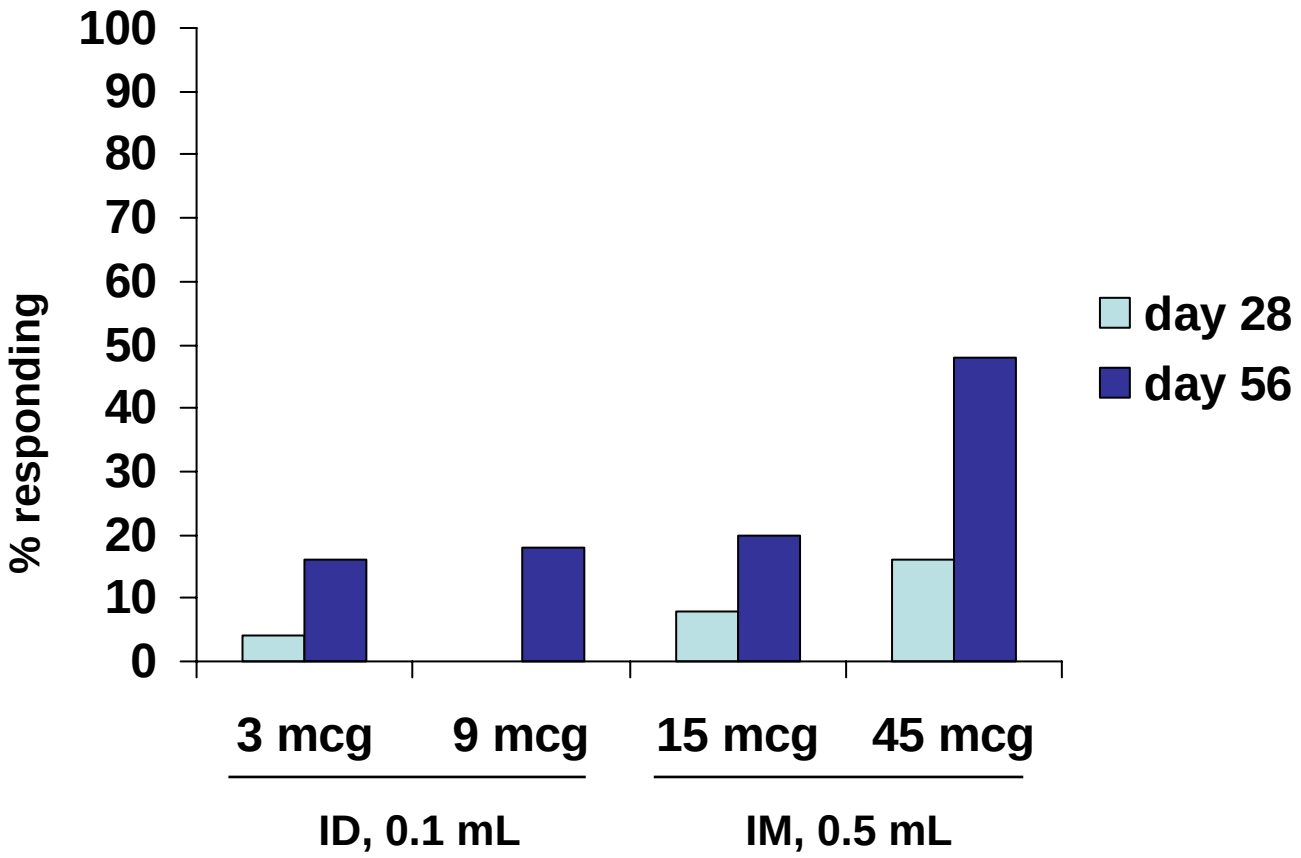
- Alternative route of administration (intradermal)
- Addition of adjuvants
- Booster doses
- Live vaccines

Intradermal vaccination with TIV: post vaccination GMT and response rate (%)



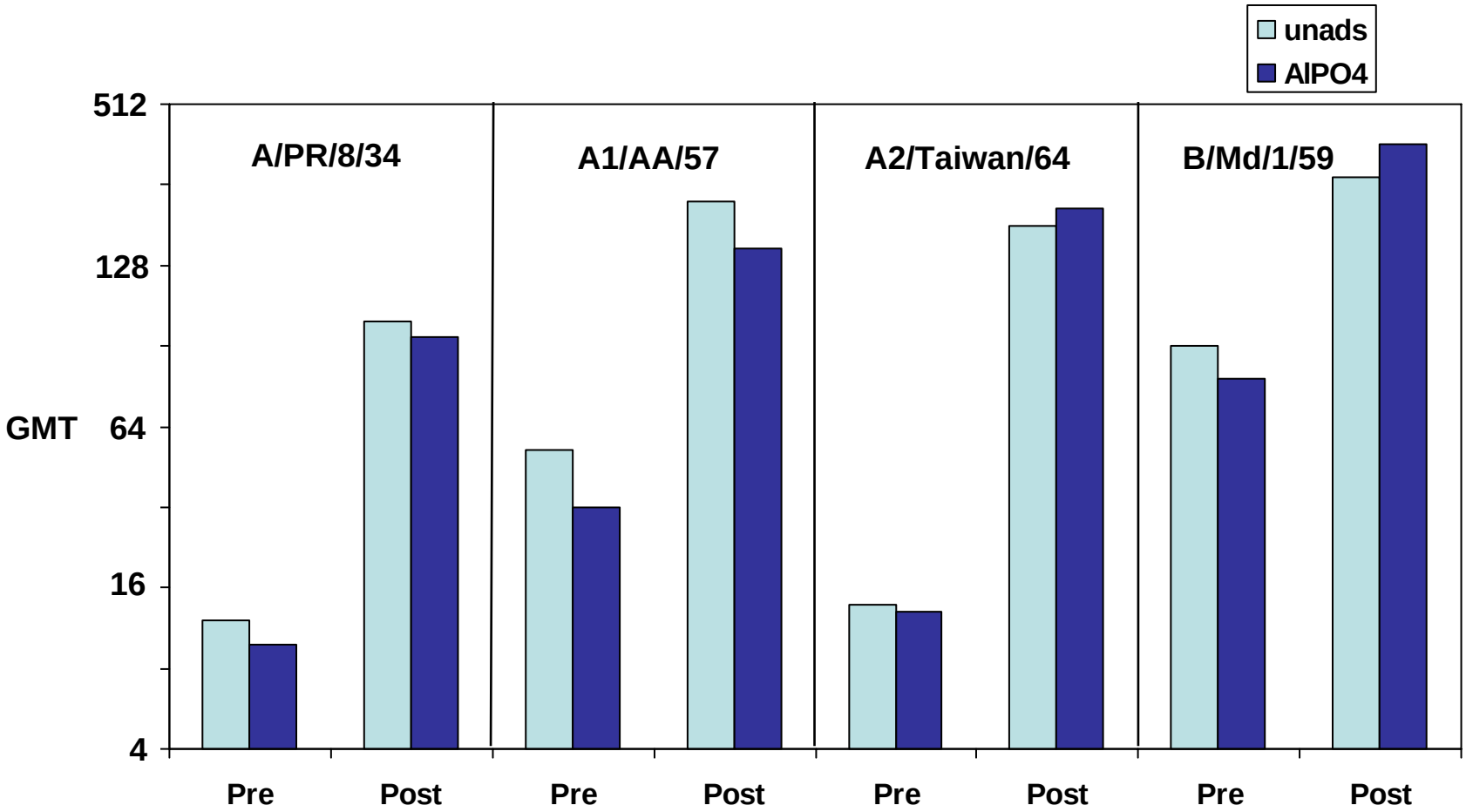
Belshe, NEJM 2005

Comparison of IM and ID route DMID 05-0015



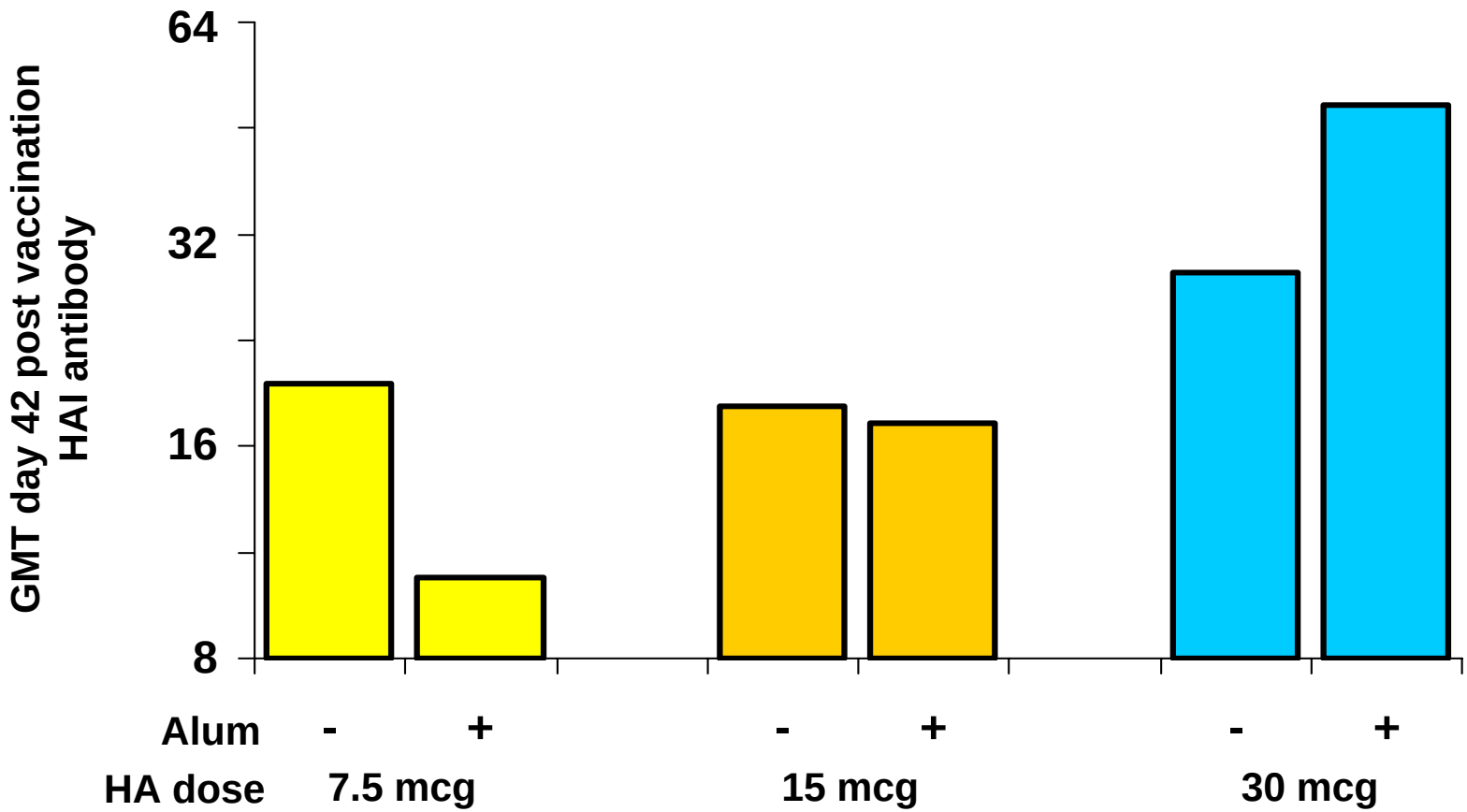
Patel, et al, 2006 ISRVI

Lack of Adjuvant Effect of $AlPO_4$ on Purified Influenza Virus Hemagglutinin in Man



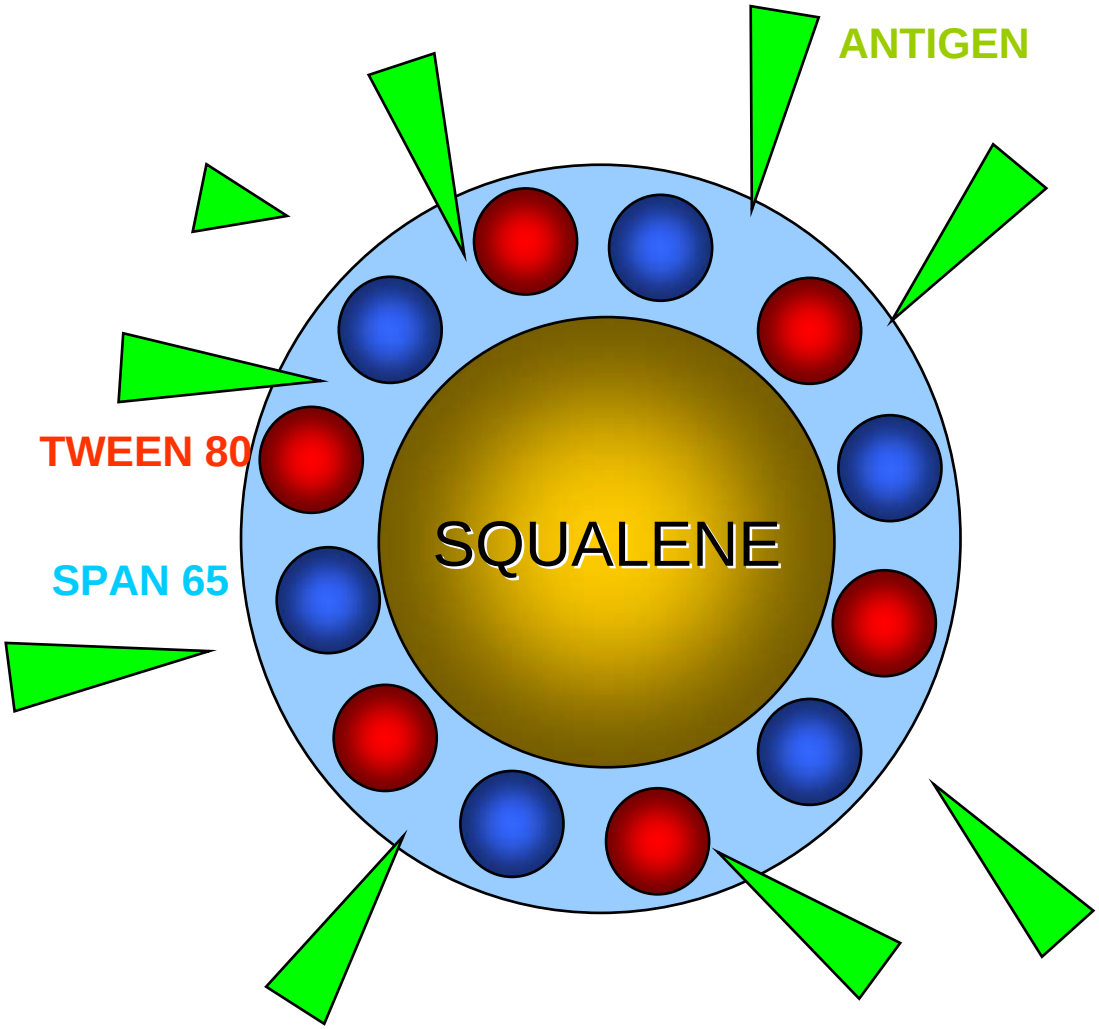
Davenport, J. Immunol 100: 1139, 1968

Effect of aluminum hydroxide on responses to A/VN/1194/04 (H5N1) subvirion vaccine



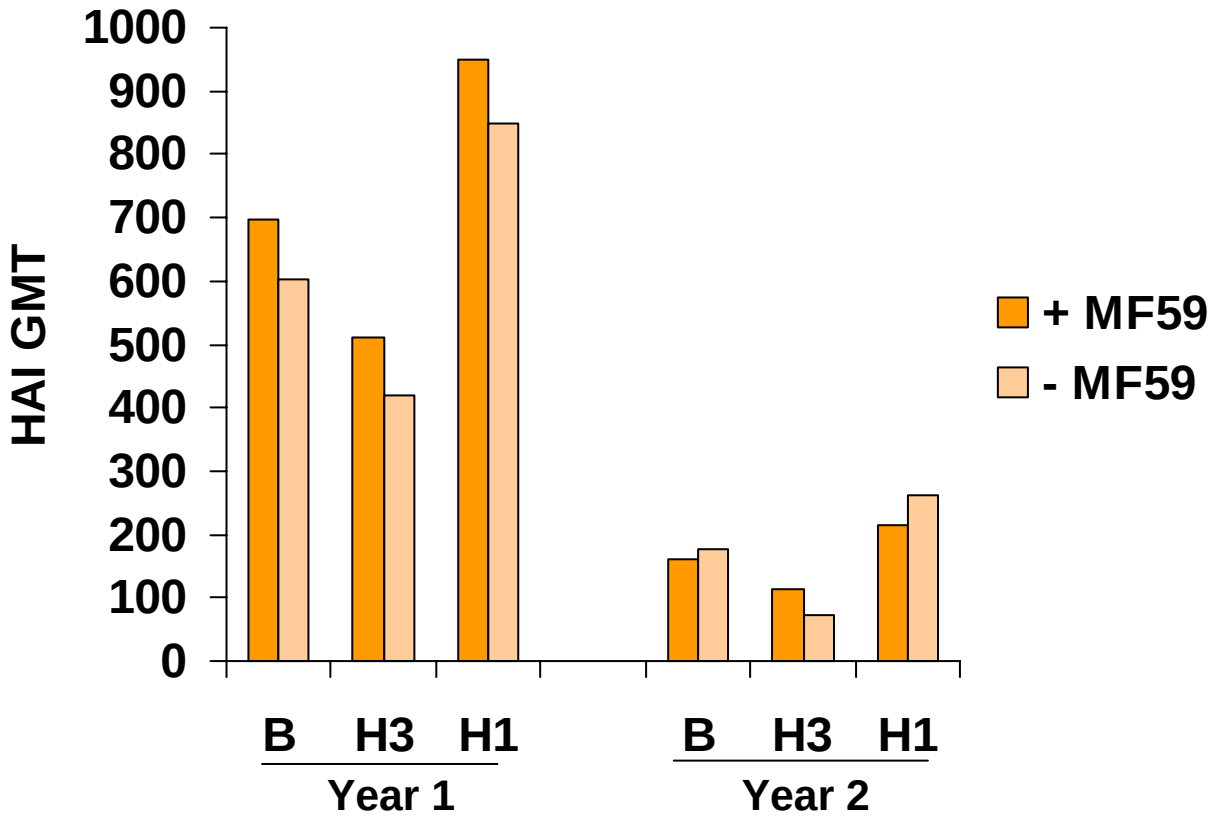
Bresson et. al. Lancet (online, May 11 2006) at www.thelancet.com

Oil-in-water emulsion – MF59



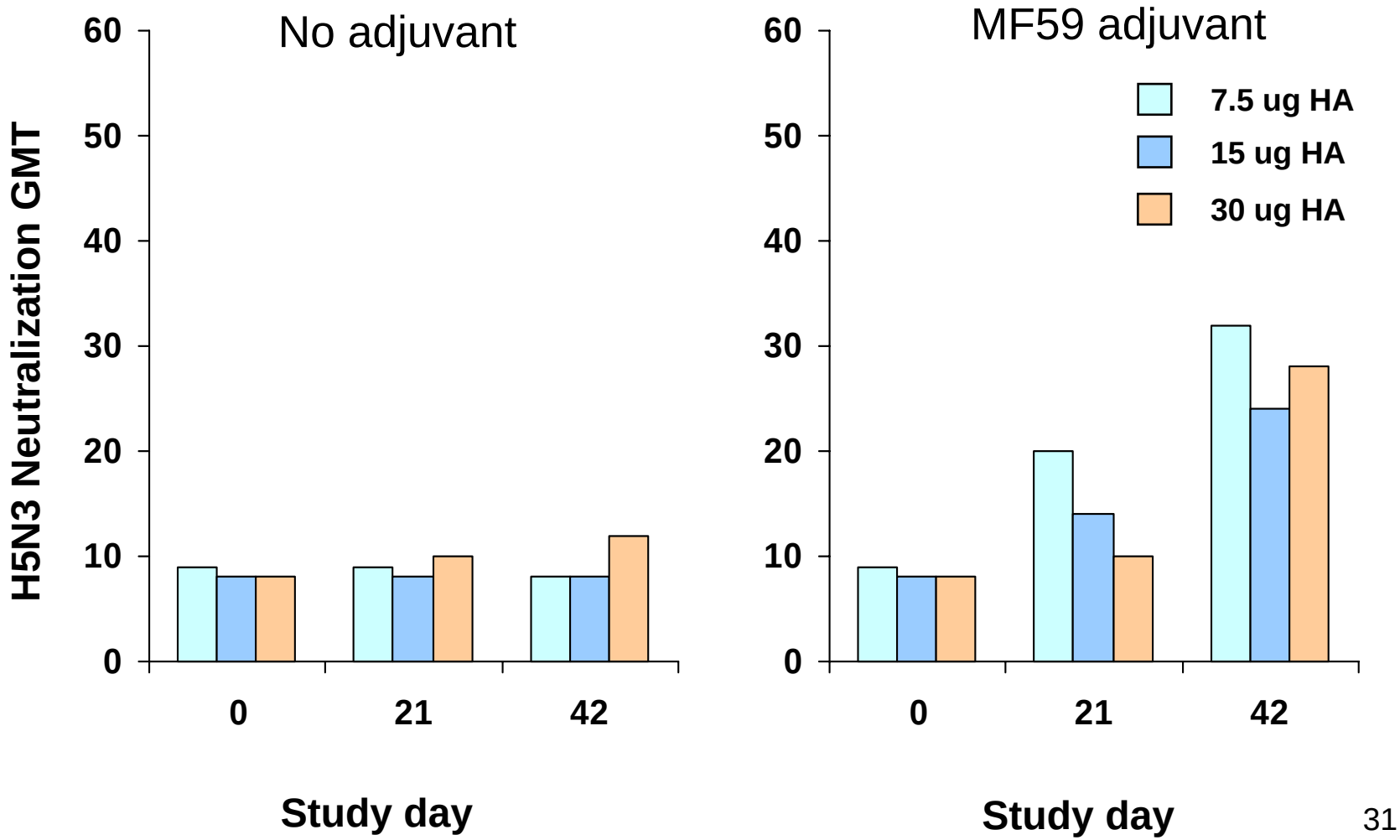
- Promising results in small studies with H5, H9 vaccine
- Increased local pain and irritation
- Licensed in some countries

Comparison of conventional TIV with and without MF59 in healthy adults



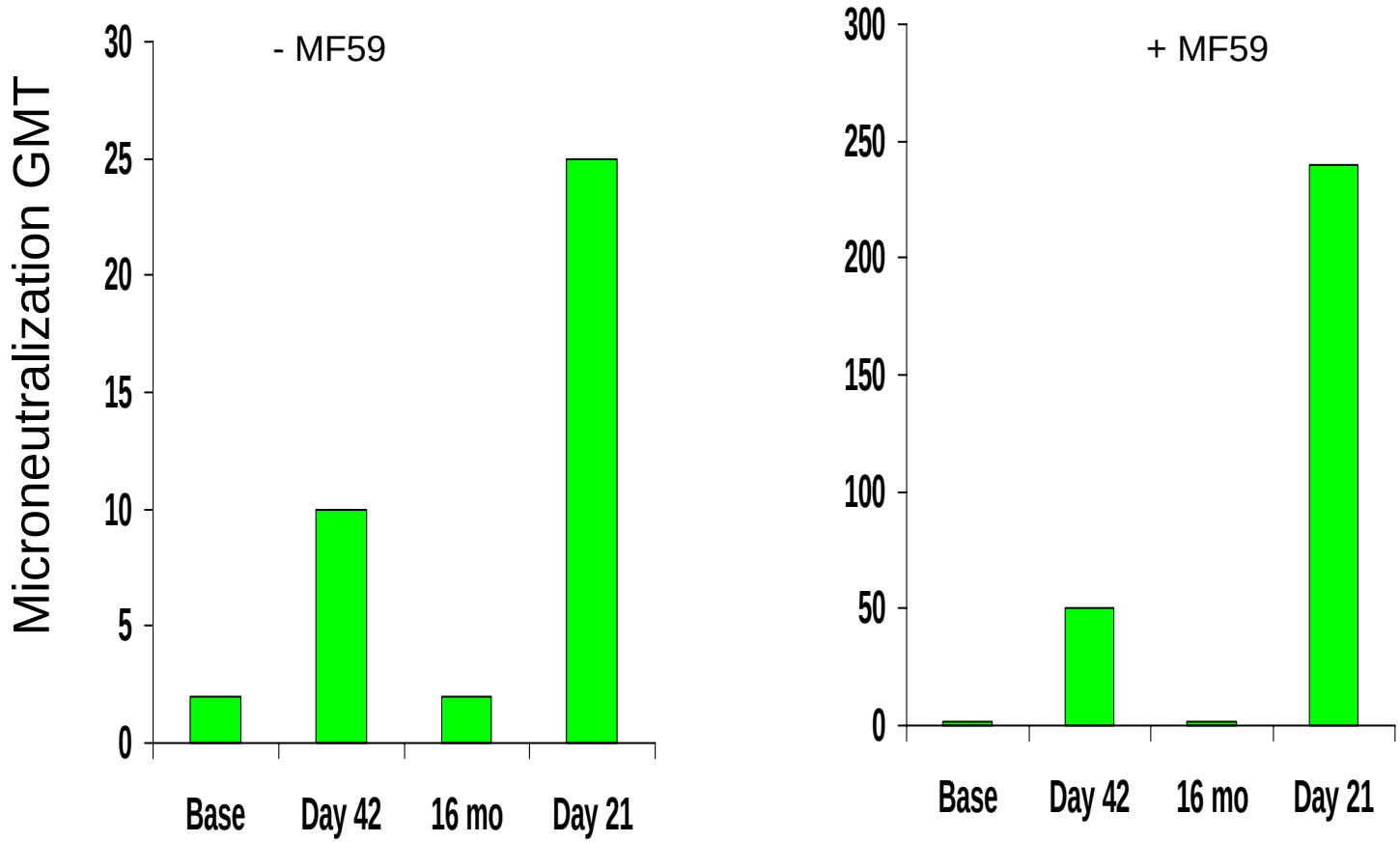
Frey, Vaccine 21:4234, 2003

Significant enhancement of the response to H5N3 virus with MF59



Nicholson *et al* Lancet 357:1937, 2001

Enhanced antibody responses following a third dose of H5N3 vaccine -/+ MF59



Stephenson *et al*, Vaccine 21:1687, 2003

Current studies being performed by DMID Vaccine Evaluation Units

Boosting strategies

05-0090	Booster dose of subvirion A/VN/1203/04 at 6 months in individuals receiving 2 doses of A/VN/1203/04	Recipients of vaccine in study 04-0063
05-0043	Single dose (90 mcg) of subvirion A/VN/1203/04 vaccine to previous recipients of A/HK/156/97 vaccine	Recipients of vaccine in study 98-012

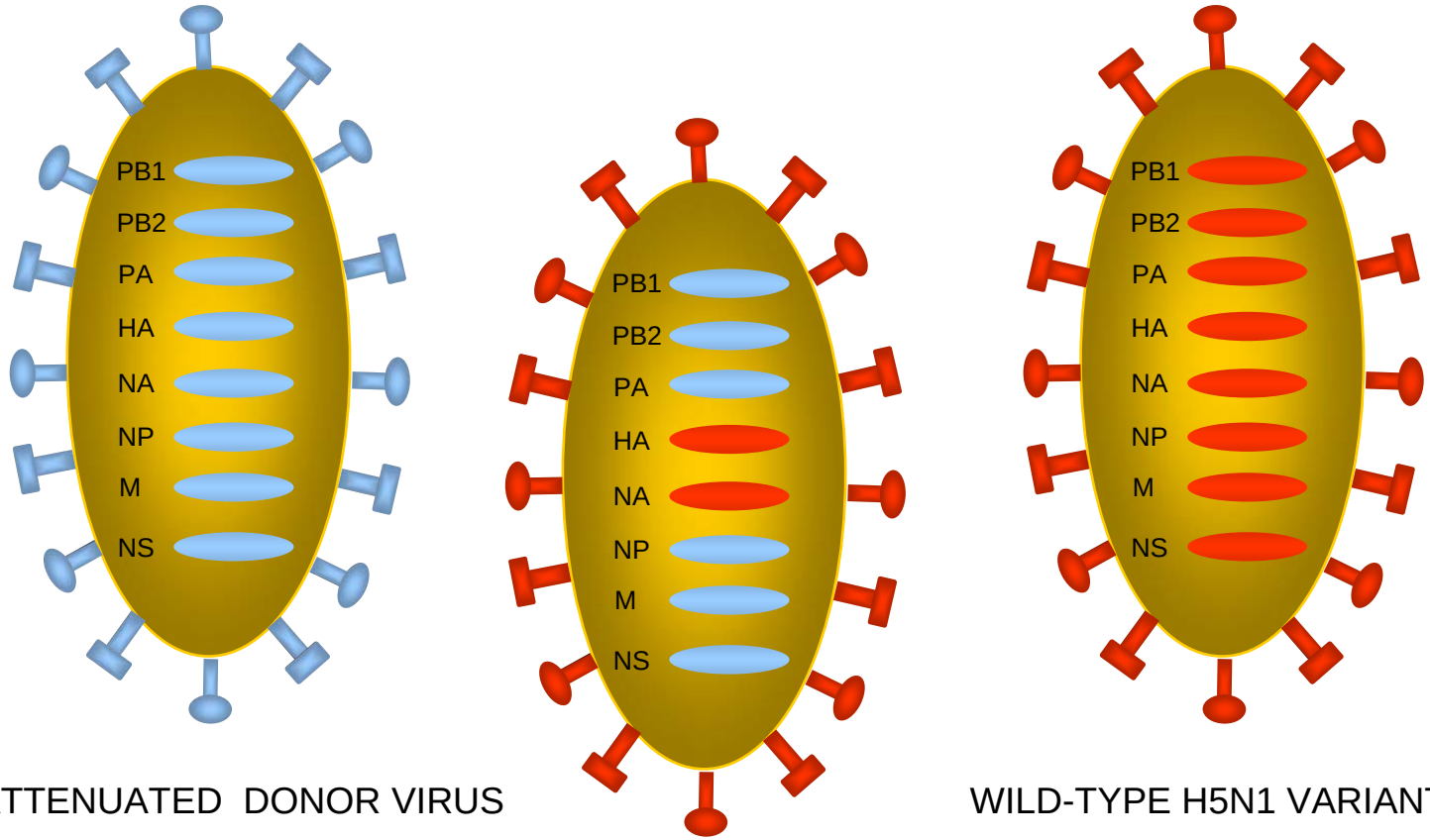
Route of administration

05-0015	Comparison of ID and IM routes of administration of A/VN/1203/04 vaccine	Healthy adults ages 18-40 years

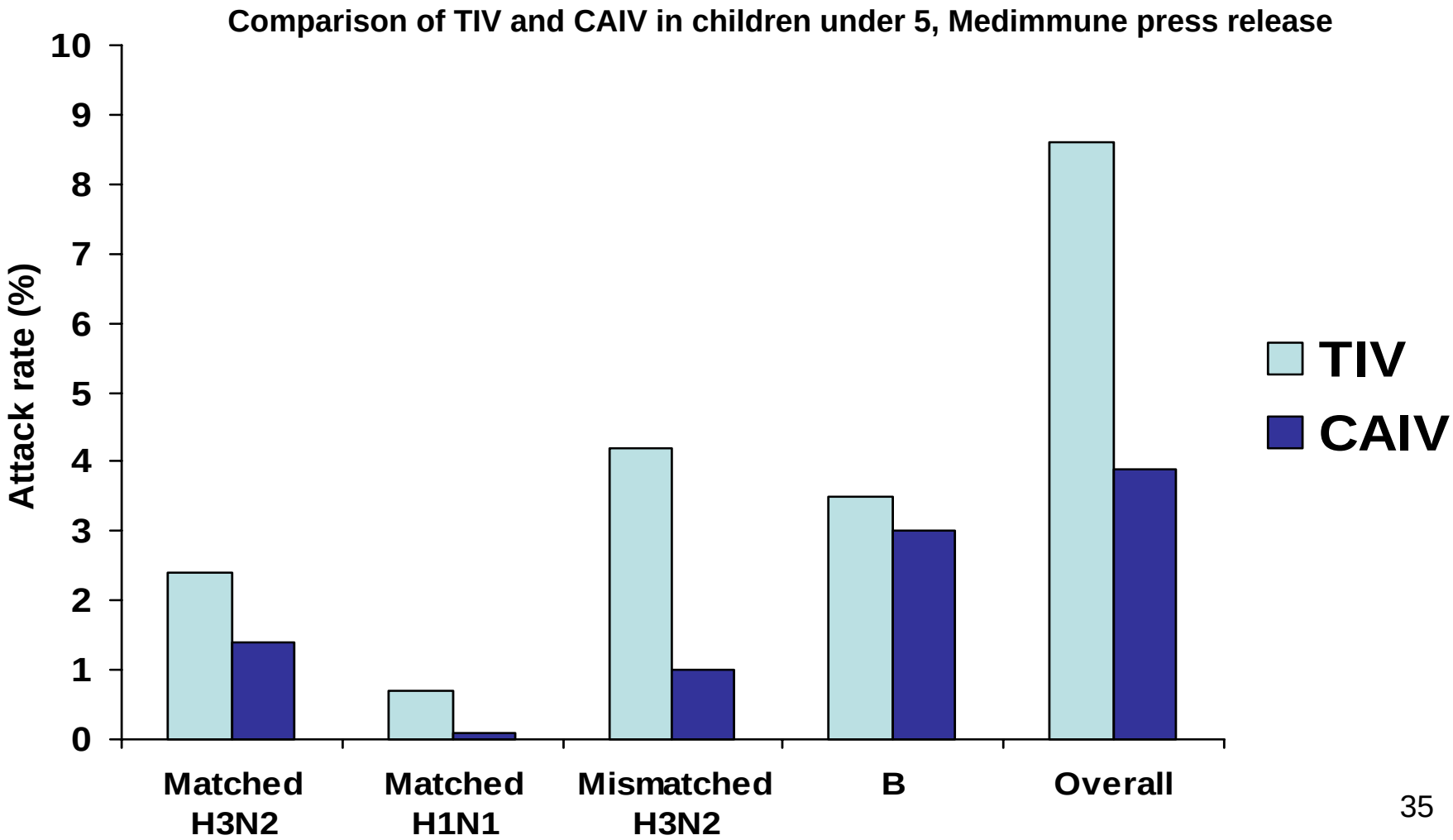
Adjuvant strategies

05-0127	Evaluation of subvirion A/VN/1203/04 at 15 mcg and 45 mcg with and without alum (500 mcg)	Healthy adults ages 18-49 years
04-062	Evaluation of subvirion A/VN/1203/04 alone (15, 30, 45 mcg) with alum (7.5, 15, 30 mcg) or with MF59 (7.5, 15 mcg)	Healthy adults ages 18-64 years

Rapid attenuation of new antigenic variants by genetic reassortment



Live vaccine is especially efficacious in unprimed, immunologically naïve subjects



Live vaccines

- Conventional CAIV are highly immunogenic in susceptible populations
  - Higher levels of protection
  - Potential use of low doses
- Induction of mucosal immunity might reduce shedding, halt transmission
- Broader cross protection

But…..

- Overattenuation is possible
- Concerns about transmission

H5N1 vaccines in development

Type of vaccine	Substrate	Adjuvant	Manufacturer
Inactivated, subvirion	Eggs	Alum	sanofi pasteur (France), sanofi pasteur (US), CSL
		Alum, MF59	Chiron (Novartis)
	Cells (MDCK)	Alum	Solvay
Inactivated, whole virion	Eggs	Alum	Biken, Denka Seiken, ID Biomedical (GSK), Kaketsuken, Kitasato Institute
		AS03	GSK
	Cells (Vero)	Alum	Baxter
Live, attenuated	Eggs	None	Medimmune

Note: for some manufacturers, detergent disruption is a component of inactivation process

H5 Vaccine: Research needs

- Understanding the correlates of H5 immunity in humans – vaccine is currently 100% effective
- Extent of cross-reactivity of antibody (e.g., with clade 2 viruses and among clade 2 subgroups)
- Development and evaluation of adjuvants and alternate routes of administration
- Understanding the factors influencing immunogenicity (antigen processing, immunodominance)
- Approaches to durable, broadly protective vaccines- CMI, innate immunity

DMID 04-063 study group

- University of Rochester: J Treanor, N. Goji
- UCLA: K. Zangwill
- University of Maryland: J Campbell
- EMMES corp: M. Wolff, H. Hill
- SRI: T. Rowe
- CDC: J. Katz
- DMID: L. Lambert, J. Hu-Primmer
- St. Jude: R. Webby, R. Webster
- sanofi pasteur: R. Hjorth

BACK-UP SLIDES

Vaccine and Treatment Evaluation Units

- Baylor College of Medicine
- Cincinnati Children's Hospital
- St. Louis University
- UCLA
- University of Maryland
- University of Rochester
- Vanderbilt University

Evaluation of live attenuated vaccines (CAIV)

- H9 and H5 candidates generated, in clinical trials
- Highly immunogenic in susceptible populations
  - Critical need to define correlates of immunity
- Potential use of low doses
  - Studies should evaluate full range
- Induction of mucosal immunity might reduce transmission
  - Development of challenge models

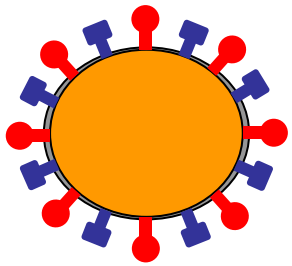
Evaluation of live attenuated vaccines (CAIV)

- Potential cross protection
  - Evaluate responses to range of antigenic variants
- Not licensed in all populations
  - Critical need to expand safety database
  - Define correlates of immunity that could be extended to elderly
- Concerns regarding transmission and reassortment
  - Clearly define conditions of deployment, expected shedding patterns, and biologic behavior of reassortants

The “Holy Grail” of Flu Vaccine:
Durable and Broadly Cross-Protective Immunity

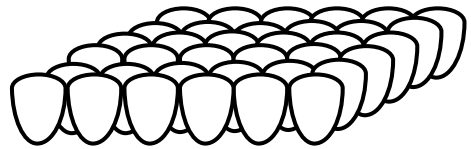
Production Of Influenza Vaccine

Fall - Winter

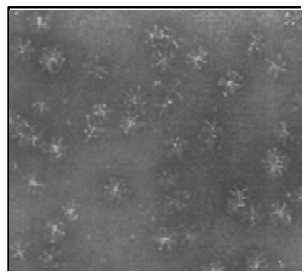


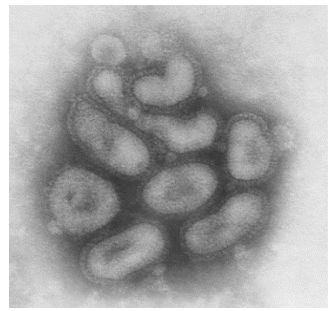
February

March - April



April - June





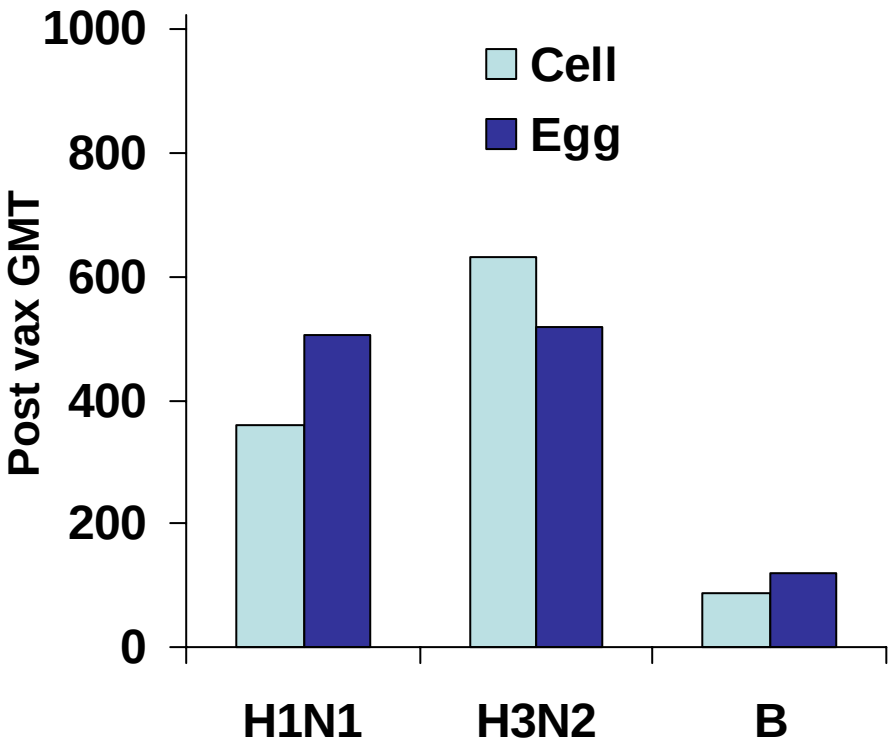
June - July

August-
September

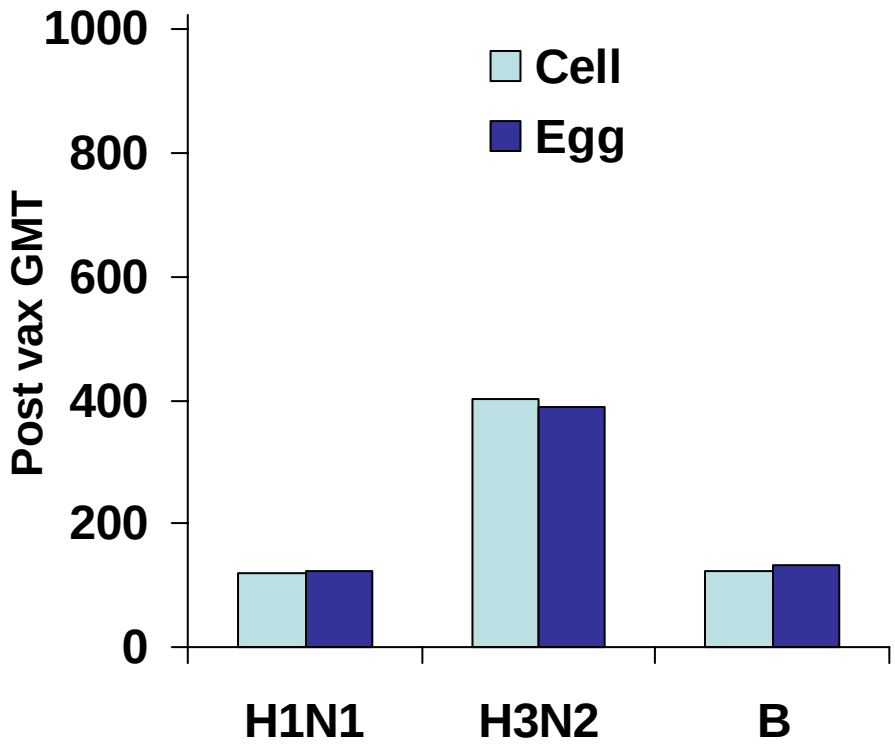
Cell culture inactivated vaccines

- MDCK cells: Canine epithelial cells
- Per.C.6: Adenovirus transformed human conjunctival cells
- Vero: Monkey kidney epithelial cells

Evaluation of MDCK cell-derived vaccine in adults



Tested against egg antigen



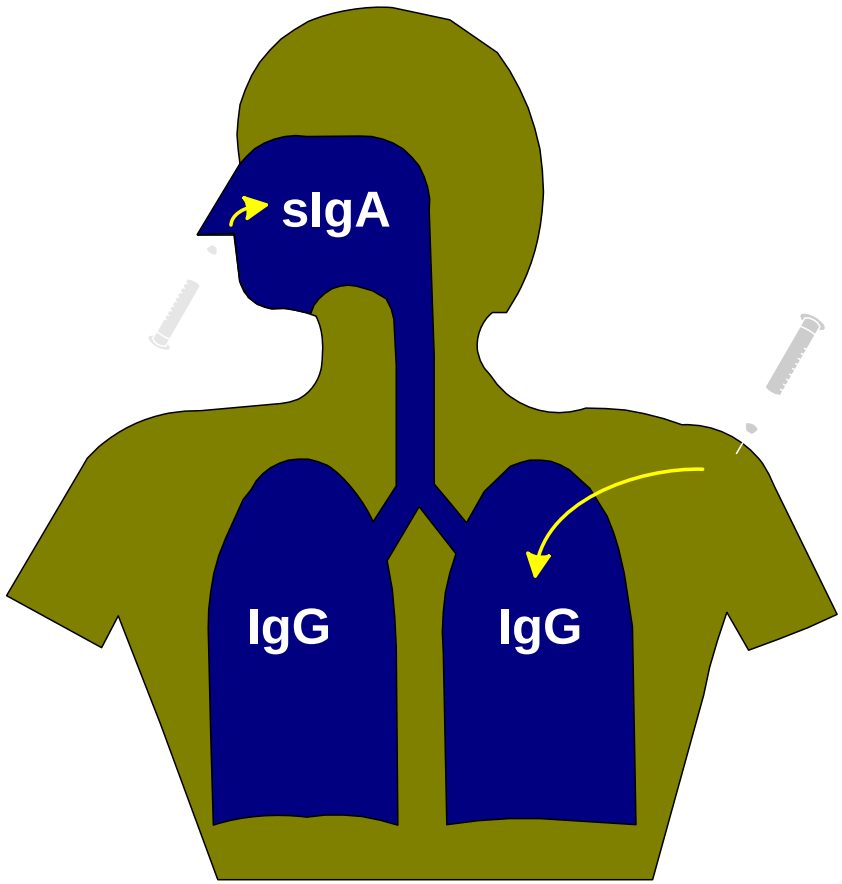
Tested against cell antigen

Halperin et al Vaccine 20:1240

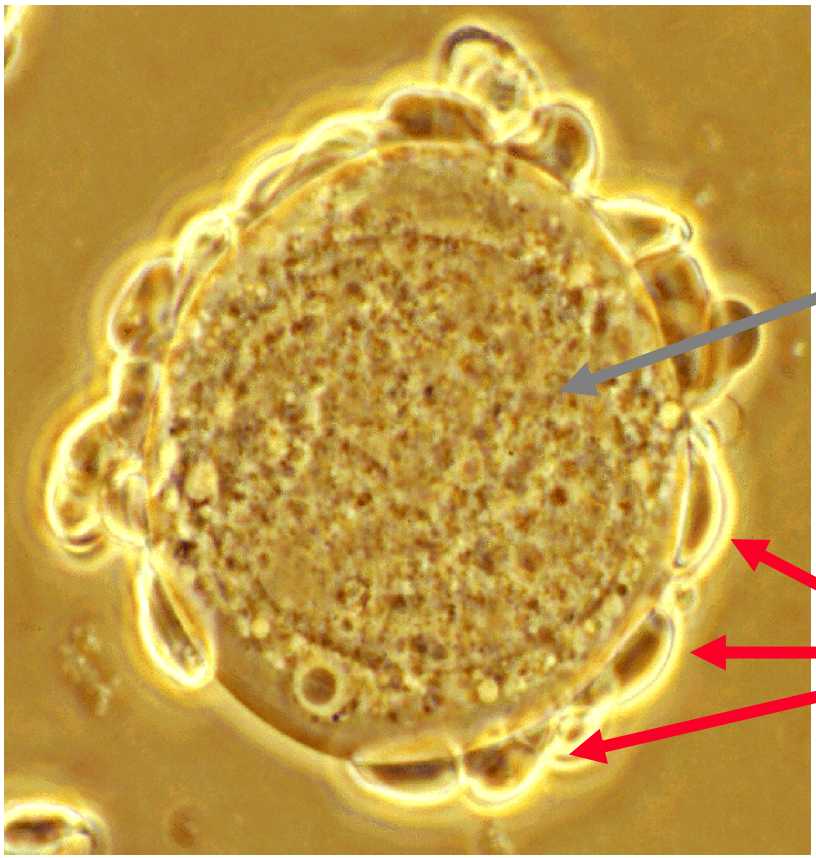
DMID 03-119 enrollment

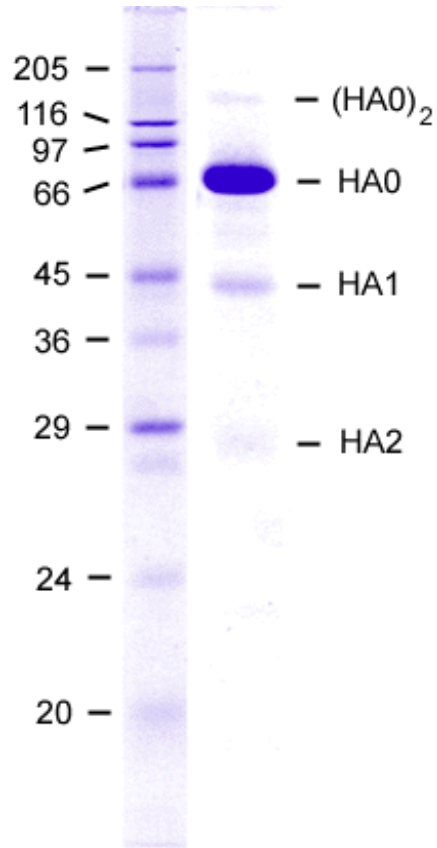
- TIV: 99 subjects, mean age=72
- 15 mcg each component (45 mcg): 99 subjects, mean age=72
- 45 mcg each component (135 mcg): 100 subjects, mean age=71
- 135 mcg each component (435 mcg): 101 subjects, mean age=71

Systemic and mucosal routes of immunization



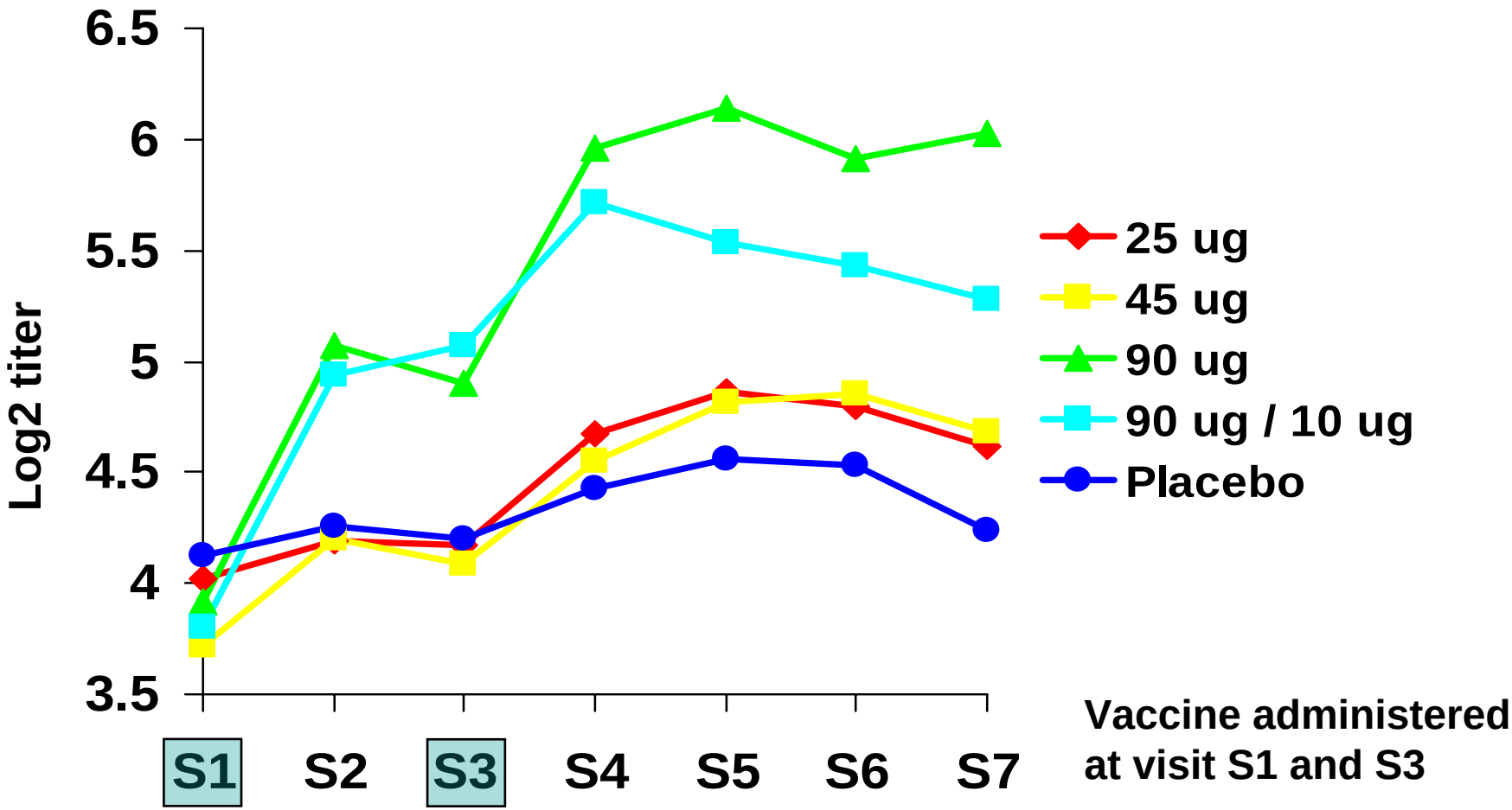
Recombinant rHA H5 Vaccine





Purified rHA H5 SDS-PAGE

Neutralization titers against A/Hong Kong/156/97

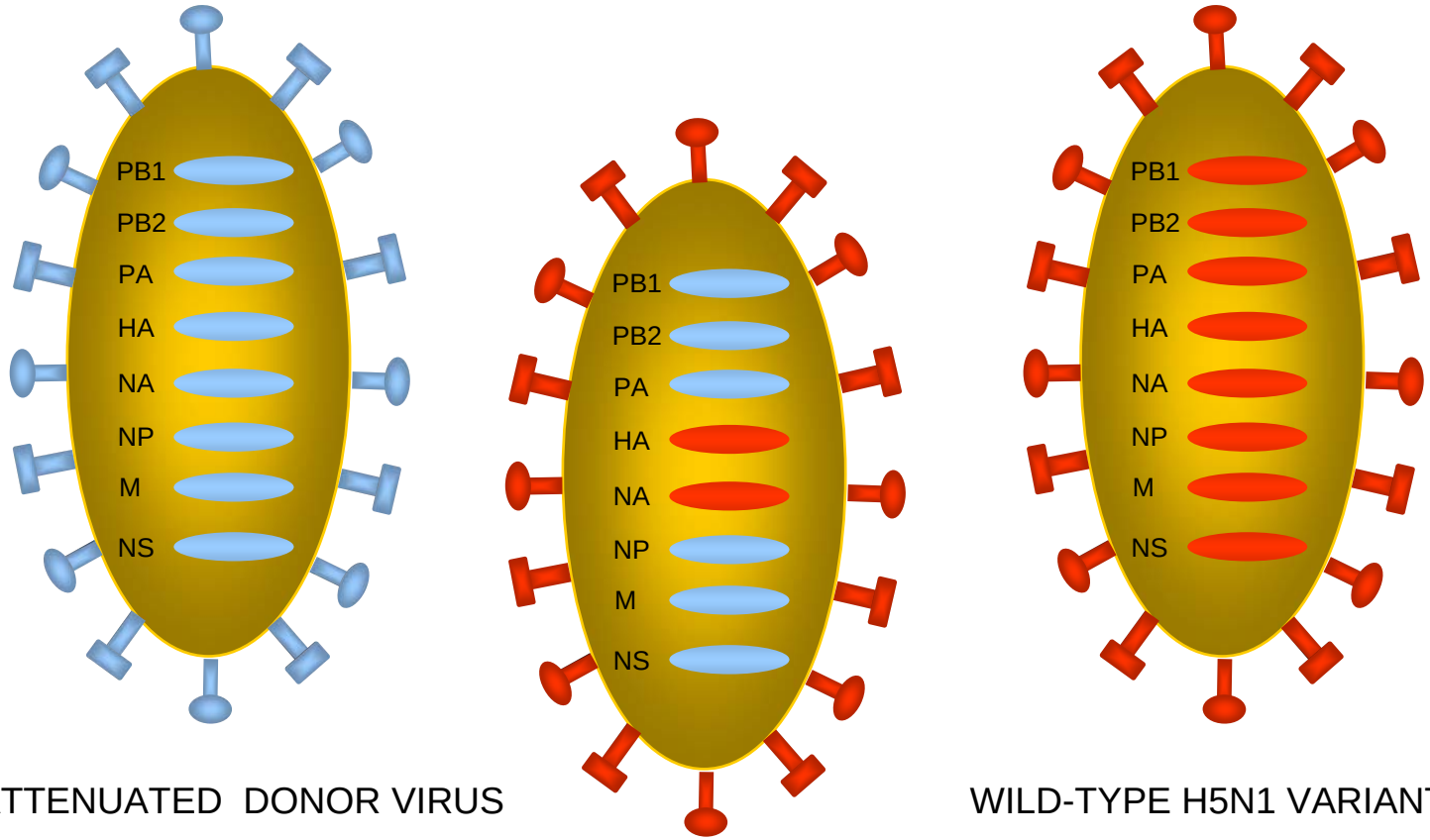


Treanor Vaccine 19:1732, 2001

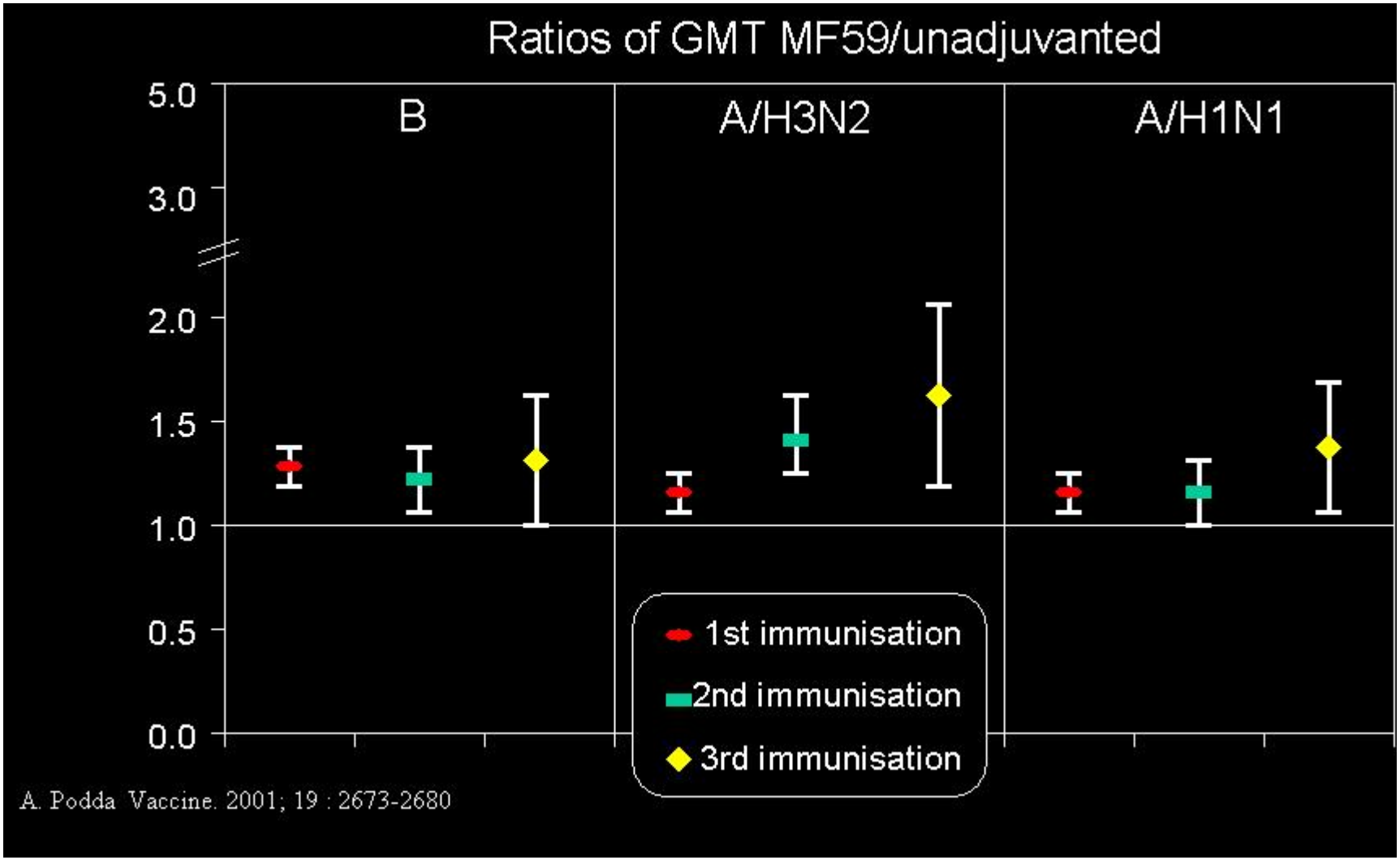
rHA pandemic vaccines

- Theoretical advantages: no need to handle biohazardous viruses, more rapid or efficient production
- Theoretical disadvantages: processing in insect cells may impact immunogenicity in naïve population
- Studies of conventional formulations in children may be useful

Rapid attenuation of new antigenic variants by genetic reassortment



Effect of MF59 on antibody responses to TIV in elderly over three seasons



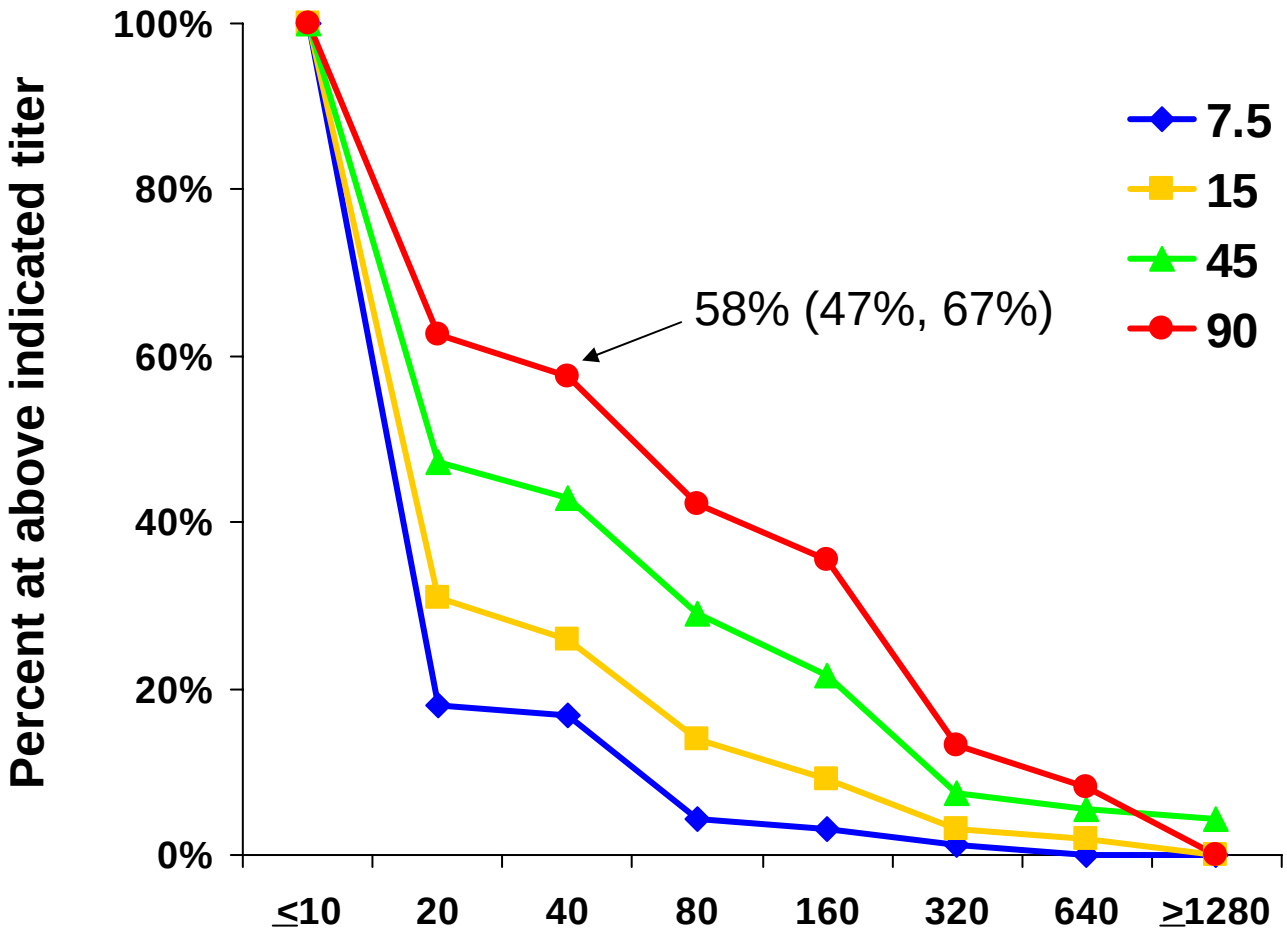
DMID 04-063 inactivated H5N1 vaccine: study objectives

- Rapidly determine the safety and immunogenicity of the candidate pandemic vaccine
- Provide precise estimates of side effect rates and immune responses
- Determine the dose-response in well controlled trials
- Generate high quality data that can be used for emergency licensure
- Gain experience with logistical issues involved in generation of a pandemic vaccine

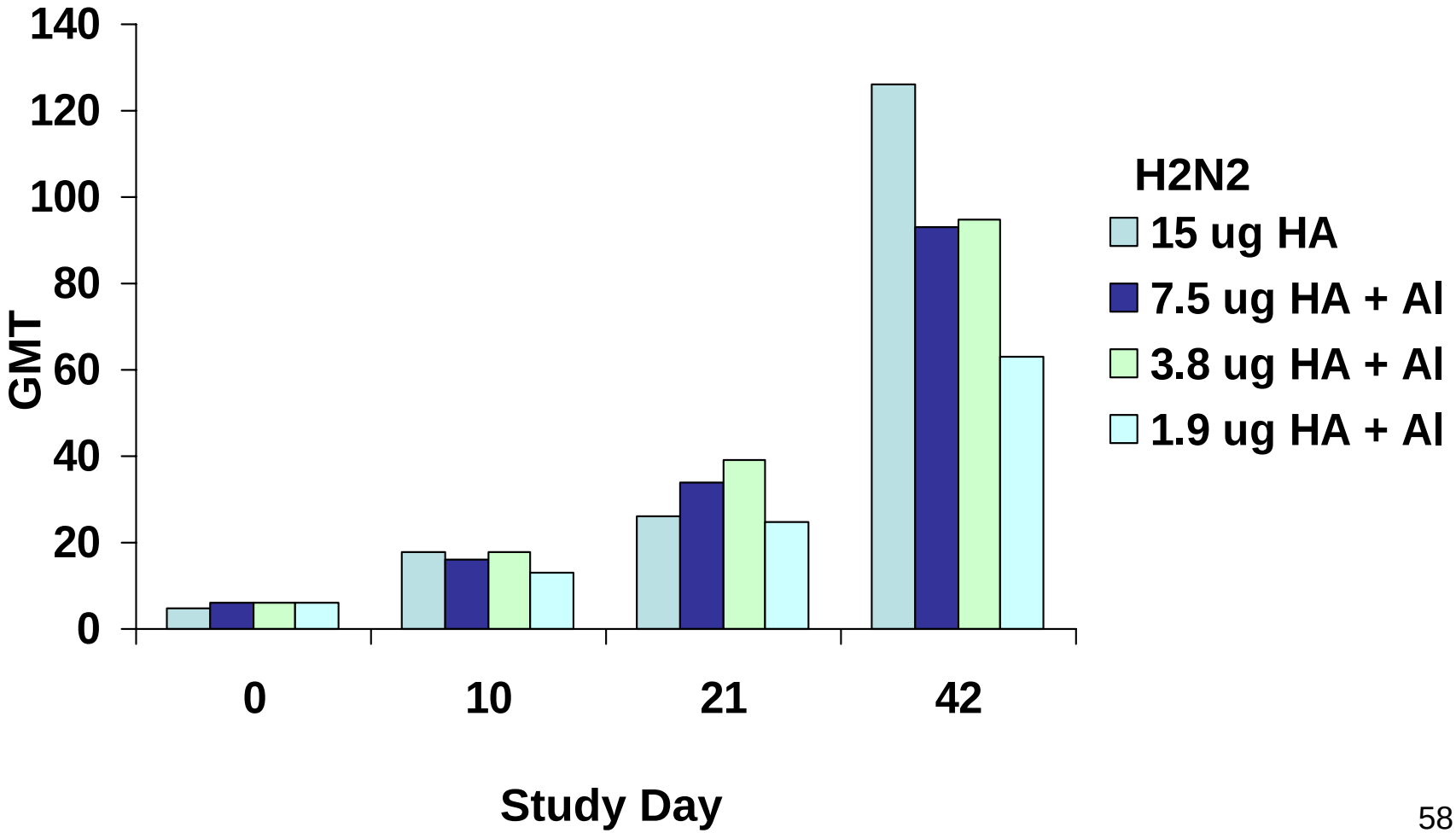
Assessment of immune response

- Microneutralization (MN) against vaccine seed virus in MDCK cells
- Hemagglutination-inhibition (HAI) against vaccine seed virus using horse erythrocytes
- Subset tested against wt A/VN/1203/04 virus (CDC, J. Katz)
- CD4 and CD8 responses to H5 peptides by elispot (T. Rock, Vanderbilt University)

Reverse cumulative distribution of serum HAI antibody titers after two doses



Alum might improve the response to a low-dose pandemic vaccine



Hehme *et al*, Med Microbiol Immunol 191:203, 2002

Comparing TIV and CAIV-T

	TIV	CAIV-T
Administration	Intramuscular	Intranasal
Immune response	Serum antibodies	Mucosal immunity
Formulation	Inactivated	Live attenuated
Efficacy children Efficacy adults <65 y	~30–70% 70%–90%	70%–90% 70%–90%
Safety	Sore arm	Runny nose
Growth medium	Chick embryos	Chick cells
Indication	Any person ≥6 mo	Healthy persons ≥5–49 y

Efficacy of live vaccines probably depends on the host

	Characteristics of cold-adapted reassortants in:		
	Children	Adults	Elderly
Safety	+++	+++	+++
Viral shedding	+++	+	+/-
Serum antibody	+++	+	-
Mucosal response	+++	++	-
Protection	+++	++	?

Efficacy of trivalent cold-adapted vaccine in children

Group	No. of subjects	No. of subjects (%) with laboratory documented: Influenza A	Influenza B	Either
Placebo	532	64 (12.0)	37 (7.0)	95 (17.8)
Vaccine	1070	7 (0.7)	7 (0.7)	14 (1.9)

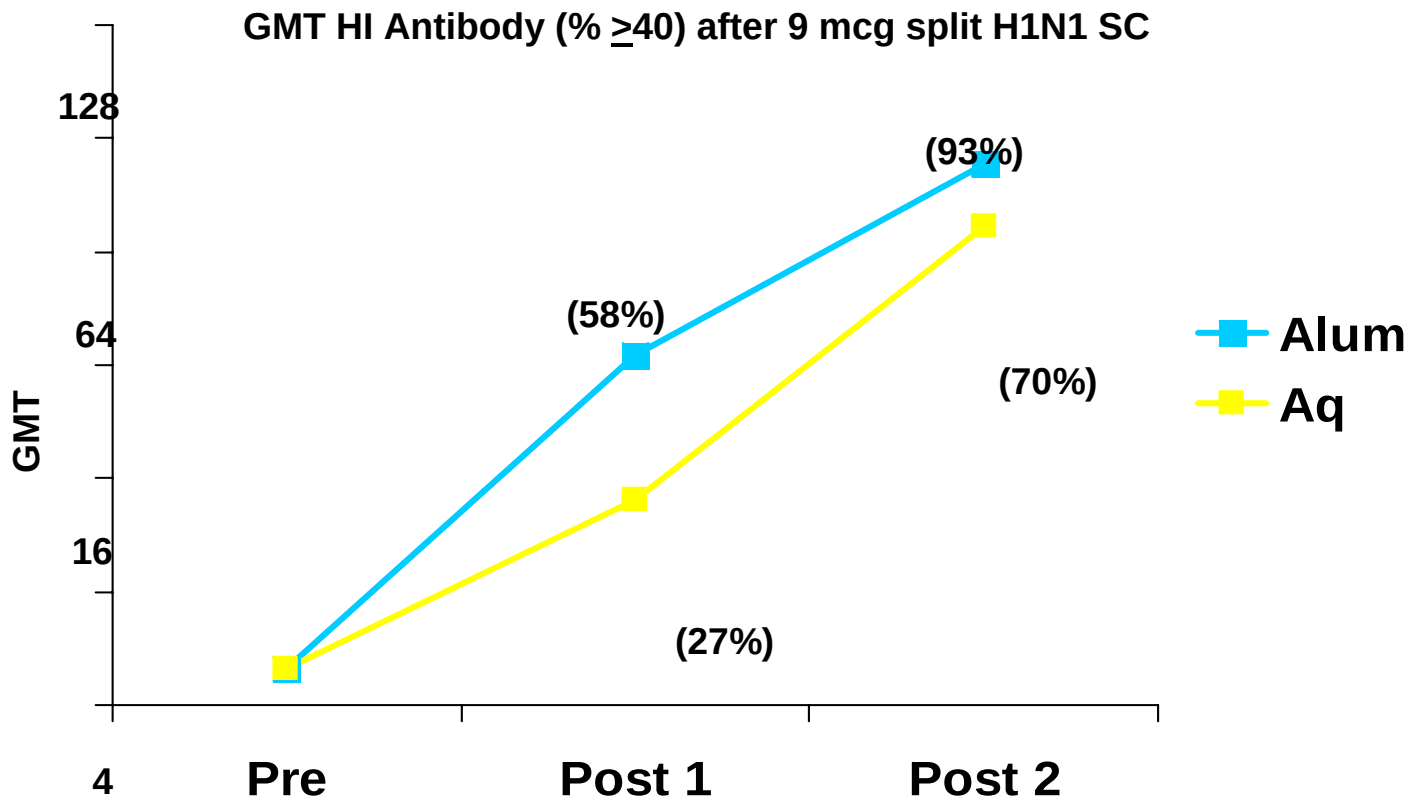
6 children in the placebo group had both influenza A and B
Protective efficacy against A is 95% (CI_{95} 88%, 97%)
Protective efficacy against B is 91% (CI_{95} 79%, 96%)

Efficacy against the drift variant, A/Sydney/95

Group	No. of subjects	No. of subjects (%) with illness due to influenza A/H3N2 viruses that were: Wuhan-like	Sydney-like	Either
Vaccine	917	0 (0)	15 (2)	15 (2)
Placebo	441	4 (1)	51 (12)	55 (12)

Protective efficacy against Wuhan = 100% (54%, 100%), efficacy against Sydney = 86% (75%, 92%)

Alum had little effect in 1977



Nicholson J Biolog Standard 7:123, 1979

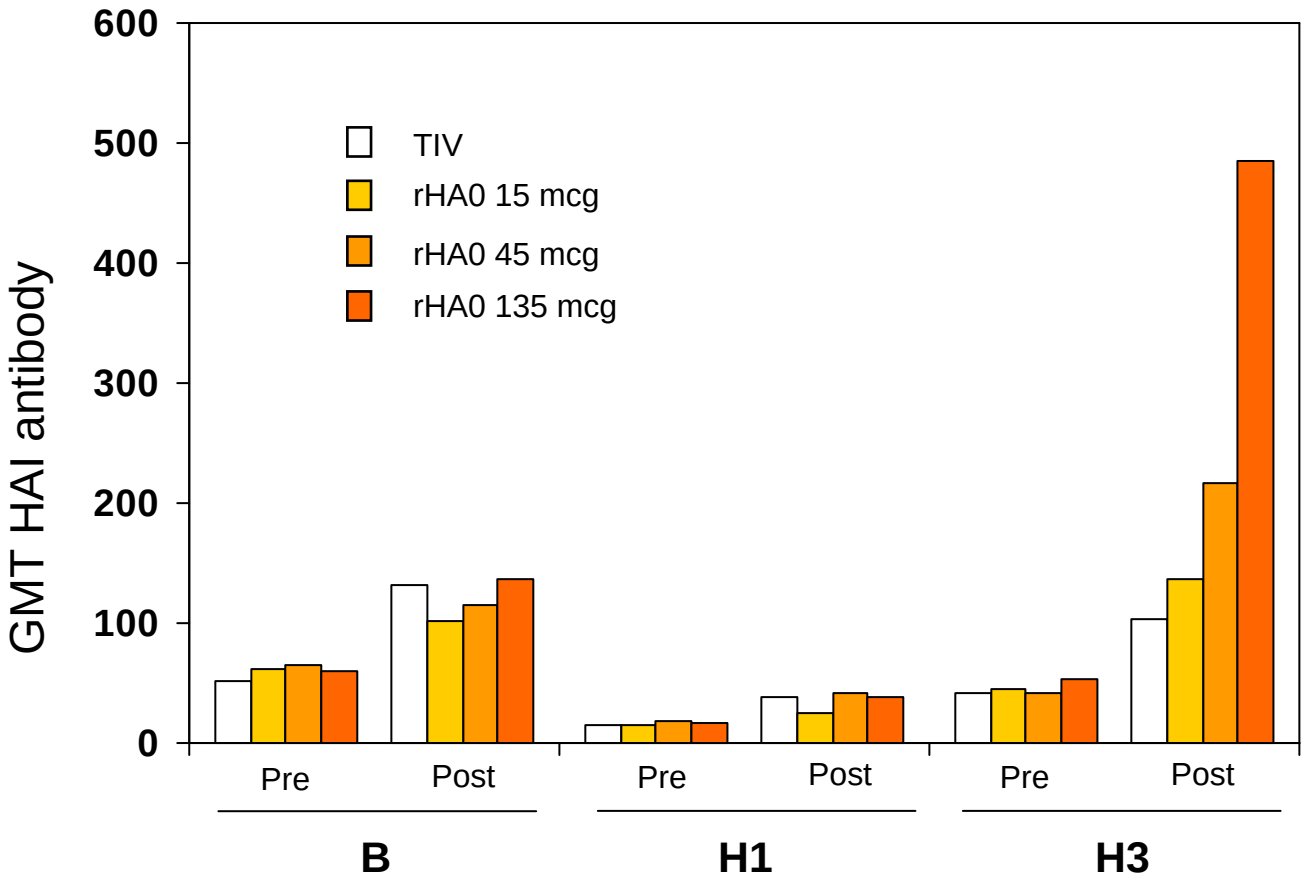
Benefits of influenza vaccination

- Reductions in disease attack rates
- Reductions in complications, antimicrobial use, medical visits
- Reduced rates of influenza and pneumonia related hospitalizations and deaths
- Reduced economic losses
- Reductions in transmission
- Reduced all-cause mortality

Evaluation of high-dose trivalent rHA_0 in elderly subjects (DMID 03-119)

- Randomized, blinded study
- Subjects: healthy adults ≥ 65 yo, stratified by previous vaccine history
- Vaccines: TIV, rHA0 15 mcg, 45 mcg, or 135 mcg/ component (435 mcg total)
- Outcomes: safety, HAI and neutralizing (NT) antibody
- Endpoint: Proportion achieving HI titer against H3 of ≥ 1:128 on day 28

Dose-dependent response to rHA0 in elderly subjects



Dose ranging rHA_0 in elderly

Serum antibody response of elderly subjects to vaccination with recombinant HA antigens or licensed subvirion vaccine.

	Proportion (n/N) achieving the following efficacy endpoints:					
	Post vaccination titer of ≥1:128 against:			4-fold or greater HI antibody response against:		
Group	H3	H1	B	H3	H1	B
135 ug/rHA	88/101	20/101	66/101	72/101	34/101	29/101
45 ug/rHA	76/99	26/98	65/99	55/99	32/98	24/99
15 ug/rHA	62/98	12/98	51/98	38/98	16/98	20/98
subvirion	49/98	21/98	63/98	33/98	37/97	34/98

DMID 03-119 conclusions

- Increasing doses of rHA0 vaccine resulted in improved HAI and MN responses to H3 component in elderly
- Dose-response relationship for H1 and B were not as clear-cut
- High dose rHA0 vaccine resulted in higher levels of antibody to H3 component than TIV
- Need studies to evaluate doses based on SRID